

Evaluation and Modification of a Micromanipulation Technique and Stubbing Method
for the Collection of Touch DNA

By

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ABSTRACT: Touch DNA is the essence of Locard's Exchange Principal at a microscopic level – when two objects come into direct contact there is an exchange of materials. Tapelifting has been shown to be the superior method for the collection of touch DNA, in part because it excludes common PCR inhibitors. This study looked into refining the tapelifting method using a modified aluminum stub that is often used in gunshot residue collection or scanning electron microscopy. This stubbing method provides barriers that reduce the risk of contamination, such as the distance between collector and evidence. Substrates that were used for collection included wood, clothing, and writing utensils. In addition, this study looked at a micromanipulation technique that involved the collection of bioparticles using a water-soluble adhesive, while being viewed under a microscope. There were modifications made to the micromanipulation protocol that was outlined by Farash et al. in their paper. One modification was the inclusion of the extraction and quantification steps. PrepFiler Express™ and PrepFiler Express BTA™ extraction kits were both used to compare any differences. The PrepFiler Express™ yielded more results, including the only full profile. The micromanipulation technique and the stubbing method both proved to be adequate methods for the collection of touch DNA.

KEYWORDS: forensic science, forensic DNA, touch DNA, micromanipulation, stubbing method, bioparticles, DNA profile

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GLOSSARY

Bioparticles	Epithelial cells, which may or may not contain nuclei. Usually composed of dead or dying keratinocytes.
Micromanipulation	Collection of bioparticles with an adhesive, visualized through a microscope
Stubbing Method	Modified tapelifting method through the use of an aluminum stub commonly used for the collection of gunshot residue (GSR) or scanning electron microscopy (SEM)
Touch DNA	Trace amounts of cellular material transferred to an object or individual through direct contact

CHAPTER 1

INTRODUCTION AND BACKGROUND

1.1 Introduction and Statement of Problem

DNA has been established as important and reliable evidence in forensic cases. It can even be deemed as the “gold standard” of evidence. DNA is unique to every individual, with the exception of identical siblings, which makes it an ideal way of characterizing who was at a crime scene. With the advancements in DNA technology and the techniques used to collect DNA evidence, smaller quantities are required and degraded samples can be analyzed with more ease. This has also made it very apparent that there are traces of DNA on many everyday objects. The first report of “touch DNA” was in 1997 when van Oorshot and Jones determined that they could collect DNA from objects that had been handled by individuals [1]. They even extended this study to show that DNA can be transferred from one individual to another with just a handshake. In the case of forensics, there could be transfer of the perpetrator’s DNA to the wrists or neck of the victim of a struggle. It is important to understand the extent of the parameters surrounding touch DNA and how this in turn affects the quality and quantity of DNA analyzed.

Common collection techniques for touch DNA include swabbing, cutting and tapelifting. However, tapelifting has been proven to be more efficient at collecting DNA than swabbing, while also omitting PCR inhibitors [2, 3]. These studies have mostly extracted the DNA by putting the entire swab head, cut

material or tapelift into the extraction tube. Farash et al. proposed a novel approach involving the use of tapelifts and the collection of epithelial cells visualized under a microscope, referred to as micromanipulation [4]. This technique has been mentioned in the literature but following a trail of references in the literature has not led to any studies using this methodology. Gunnarsson et al. [3] mentioned the technique and included a reference, which led to Hall and Fairley [5] who also only mention the technique, resulting in a dead-end.

Research into the validation of the techniques used by Farash et al. is important to determine the efficiency of the method. In addition, the DNA in this study was quantified, although Farash et al. [4] and Wickenheiser [6] advise against the quantification of touch DNA samples because it would be a waste of already minute quantities of sample. The quantification step will be included as it is mandatory in a forensic laboratory, therefore the methods as they apply to real casework should be assessed [7].

For these reasons, a study looking at the advantages and disadvantages of a procedure using micromanipulation techniques and modified aluminum stubs, as a tapelifting method, would be of interest. This serves as an evaluation of the methods proposed by Farash et al., while modifying the protocol to closer match the standards in a real forensic laboratory. This includes extracting and quantifying the amount of DNA in a sample, which was not done in the Farash et al. study, but required in casework [4, 7].

1.2 General Background

1.2.1 Touch DNA

Every day humans shed over 400 000 epithelial cells [2, 6, 8]. When an individual makes contact with an object, these epithelial cells are deposited, such as stated by Locard's Exchange principle, which says that when two objects come into contact there is an exchange in materials [9]. "Touch DNA" is biological evidence that is left after an individual makes contact with a substrate [4, 6, 10]. There are many factors that determine the amount of touch DNA that can be collected, such as the substrate on which the DNA is deposited, the amount of time the object is handled, and what is known as "shedder type" [6].

It has been shown that a more complete touch DNA profile can be obtained from porous objects (i.e. fabrics), rather than non-porous substrates (i.e. glass) [6, 10]. This is the opposite from the ideal substrates for the collection of fingerprints. The reasoning behind this common finding is that the cells, and hence the DNA within those cells, can become embedded within porous substrates [11] . On a non-porous substrate, the DNA is exposed to environmental factors and can be wiped off or transferred to another object.

An individual can be classified as a "good" or "bad" shedder, wherein a good shedder would leave a full DNA profile after physical contact with an object, while a bad shedder would leave fewer cells and thus fewer detected alleles [6, 12, 13]. The parameters that classify an individual as a good shedder was characterized by Lowe et al. when they said that a good shedder is an individual who leaves a full DNA profile 15 minutes post-hand washing [14]. Another study

brought shedder status into question and proposed that an individual can be a good or bad shedder depending on the time of testing [15]. In terms of an actual crime scene, the shedder status of the assailant will not be known when examining touch DNA evidence. Therefore some studies including Daly et al. [15] have decided not to determine the shedder status of their volunteers, while still acknowledging this common classification.

1.2.2 Modified Tapelifting Technique

As previously mentioned, there are a few well-established techniques when collecting DNA evidence. However, the tapelifting method has been shown to be more effective than swabbing or cutting methods [2, 3, 10]. Even when objects that are known to contain PCR inhibitors (e.g. denim and leather) are tested using tapelifting as the collection method of choice, there was no indication that PCR inhibitors were included in the analysis [2, 3]. There was even success in collecting DNA with tapelifts from sunflower seeds, when the swabbing method yielded no results [2]. Overall, throughout the literature it has been noted that tapelifting provides a more complete DNA profile, rather than the more common swabbing methods. Hansson et al. [16] compared one minitape to three different types of swabs, and had better DNA results with the minitape over all three swab types. Verdon et al. [10] looked at two different types of tape as well as one swab, and both tapes worked better than the swab. These studies extracted the DNA by putting the entire tapelift into the extraction tube.

There were a few references in the literature, wherein the authors recognized that skin cells were also being collected with gunshot residue (GSR)

evidence from suspect's hands [5, 17]. One of these papers dated back as far as 1996 [17], and the other was published in 2012 [5]. However, there was one paper from the Netherlands that noticed the convenience of the aluminum stub for DNA collection on its own [13]. The authors noticed the ease at which the stub could be handled during collection, as well as the uniform pressure that could be applied to the area of collection [13].

1.2.3 Micromanipulation

All of the common collection methods that are used for instances of touch DNA are performed “blindly” on the sample [4]. This is to say that the analyst does not actually know whether there is trace DNA evidence present in the area that they are sampling. This blind collection of DNA can displace epithelial cells from one individual that were in a distinctly different location than the epithelial cells from another individual [4].

A novel method is the microscopic examination of the adhesive before the analysis process. The only brief mentions of this method that were found in the literature were from laboratories located in central Europe, including the Netherlands [13], Sweden [3] and the United Kingdom [5]. Few laboratories actually use this method as it is deemed time-consuming. This procedure was clearly outlined by Farash et al., whereby they mounted the tapelift onto a microscope slide, which could be optionally stained to aid in the visualization of epithelial cells [4]. This method provides numerous advantages in place of its obvious disadvantage: time. Before the extraction process has even begun, the collection of biological material can be confirmed under the microscope.

1.3 DNA Extraction Kits

There are two DNA extraction kits that are used with the AutoMate Express™ Forensic DNA Extraction System: PrepFiler Express™ Forensic DNA Extraction Kit and PrepFiler Express BTA™ Forensic DNA Extraction Kit. The PrepFiler Express™ extraction kit is suited for samples such as bodily fluids on a variety of different substrates, such as cotton swabs or fabrics [18]. The PrepFiler Express BTA™ extraction kit is used for bone, tooth and adhesive samples [18]. Both extraction methods use dithiothreitol (DTT) in the lysis buffer solution. However, the PrepFiler Express BTA™ extraction kit also uses Proteinase K (ProK) in the extraction process. These two extraction kits will be used for the samples in this study, to compare whether the PrepFiler Express BTA™ extraction kit provides an advantage because the epithelial cells will be entering the lysis buffer solution by way of a water-soluble adhesive.

1.4 Goals of the Study

There have been numerous studies that discuss touch DNA evidence and comparison of the collection methods. It has been shown on numerous occasions that tapelifting as a collection method provides many advantages over the swabbing or cutting methods available. This study plans to further the research in the field of tapelifting as a collection method, while also validating the results and conclusions obtained by Farash et al. [4] That study, along with many others, did not quantify the amount of DNA in the sample prior to amplification. Thus the goals of this study are as follows:

1. Confirm the results obtained by Farash et al. using microscopic examination of the tapelift before extraction [4]
2. Assess the implication of quantifying the DNA before PCR amplification
3. Compare the results obtained by using the PrepFiler Express BTA™ extraction kit and the PrepFiler Express™ extraction, since a water-soluble adhesive is being used

CHAPTER 2

MATERIALS AND METHODS

2.1 Selection of Aluminum Stub

The first step was to determine the best aluminum stub in terms of adhesion, size, and convenience. The two aluminum stubs that were tested were from TriTech Forensics (Southport, NC) and Ted Pella (Redding, CA). The stubs from TriTech Forensics were GSR stubs that were already prepared, i.e. the pin was a part of the mount. The pin also already had its own black, carbon adhesive. The Ted Pella stubs were SEM stubs that required preparation, by inserting the pin into the mount, using 45° angle tweezers. The aluminum pin did not have its own adhesive.

Many preliminary trials were done with the TriTech Forensics stubs in order to test the adhesion of the Gel-Pak film (WF-40-X8-A) (Hayward, California) to the stub and the adhesion of the sample to the Gel-Pak film. The Gel-Pak film was used in the Farash et al. study, which was the reasoning for this choice of tapelift. Ultimately the black, carbon adhesive proved to be a disadvantage, since it could pick up DNA if the whole stub was not covered by Gel-Pak film. This would result in DNA being collected but not tested. The Ted Pella stubs were better for the purposes of these techniques, even though putting the pin in the mount added to the time of their preparation. It was also decided that the Gel-Pak adhesive backing would not be in direct contact with the aluminum stub. Rather, double-sided tape would be used to hold the Gel-Pak onto the stub.

2.2 Selection of Double-Sided Tape

Once the aluminum stub had been chosen, the next step was to select the type of double-sided tape that would be used to hold the Gel-Pak film onto the stub. There were three double-sided tapes that were tested, and chosen because they were already in the lab: 3M Scotch® Mounting Tape (St. Paul, MN), Scotch® Wallsaver Removable Poster Tape (St. Paul, MN), and Crafts Double Sided Tape (Dollarama; China).

A square measuring 0.9cm x 0.9cm of each type of tape was placed onto one of the pinheads of the stub. Then a 1.0cm x 1.0cm square of the Gel-Pak film was placed on top with the white backing still in place. The Scotch® Wallsaver Removable Poster Tape was noticeably not sticky enough to hold the Gel-Pak in place and was not chosen for this reason. Both the Crafts and 3M Scotch® Mounting tape were sufficient in holding the Gel-Pak in place during sampling. The 3M Scotch® tape included a thick foam layer, which did not include any benefits; for this reason the Dollarama tape was chosen.

2.3 Preparation of Aluminum Stubs for Tapelifting

The aluminum stubs for DNA collection were made in-lab. A 0.9 cm x 0.9 cm square of double-sided tape (Dollarama; China) was cut out and placed on the aluminum pin of the SEM stub. The pinhead was 1.27cm in diameter, which allowed for most of the pinhead to be covered. The modified stub was able to be stored at room temperature until the next step with the clear, protective layer still in place on the Gel-Pak film. When ready, the clear protective cover was removed

with a pair of forceps. Next, a square of 1 cm x 1 cm Gel-Pak film was placed on top of the double-sided tape with the white backing still on. The clear plastic cover was left on the adhesive until ready for sampling. The stub's plastic vial was also used to cover the aluminum pin until ready for sampling.

2.4 Collection of DNA

Three substrates were chosen for this study, which included wood, clothing and writing apparatuses. The writing apparatuses were pens (Papermate®; Oak Brook, Illinois) and pencils (Staples® Simply Wood). The female author of this paper deposited alleged touch DNA on the substrates through handling of the objects or by wearing them, in the case of the clothing. The collection method was the same for all three of the substrates. When ready, the plastic vial of the stub was removed. Using sterile forceps, the clear, plastic protective layer was removed from the Gel-Pak film. Similarly to the collection of GSR evidence, the stub was used to sample DNA by pressing the adhesive onto the sample and lifting. This was done either until the adhesive was no longer sticky, which occurred often with the clothing and the wood, or until the entire surface of the substrate had been sampled for touch DNA. The plastic vial was put back on the stub after collection.

Once the touch DNA had been allegedly collected onto the adhesive, the stubs were brought into the extraction lab for microscopic confirmation that bioparticles had actually been collected. The plastic vial of the stub was removed, and using a pair of sterile tweezers, the Gel-Pak film was removed from its white backing, which remained adhered to the double-sided tape on the stub. The Gel-

Pak film was then placed onto a microscope slide, and carefully pressed into place with the tweezers at only the edges and corners.

Once the slide was prepared, it was visualized using a Swift M3-F Forensic Comparison Microscope (Carlsbad, California) to ensure that epithelial cells were collected during the collection process. The cells could be visualized at either the 40X or 100X magnification. The slide could be stored at room temperature in a microscope slide box until ready for micromanipulation.

2.4.1 Micromanipulation of Biological Material Collected

Once there was confirmation that there had actually been bioparticles collected on the adhesive, the bioparticles were collected for analysis. Farash et al. provided an option in the protocol for staining the slides with Trypan Blue, but it was not used in this study. For the PrepFiler Express™ extraction process, a PrepFiler™ LysSep™ column was used without the hingeless sample tube. The column portion was filled with 220µL of lysis buffer, which is only a portion of the total 500µL required for extraction. This column was placed onto a microscope slide with a piece of double-sided tape to hold it in place. For the PrepFiler Express BTA™ extraction, 220µL was the total volume of lysis buffer solution required in the extraction process, so only 150µL was used inside the column.

Next, another microscope slide had a piece of double-sided tape attached to it, with a piece of 3M™ Water-Soluble Wave Solder Tape 5414 (St. Paul, Minnesota) added on top with the adhesive side facing up. Using a tungsten needle (The McCrone Group; Westmount, Illinois) the top layer of the solder tape was

gently scraped in order to form a ball of adhesive on the end of the needle. This was done at 40X magnification. The needle was removed from under the microscope and placed in a rack.

The slide containing the collected bioparticles on the Gel-Pak film was placed on the stage in the place of the slide with the solder tape. Visualizing the slide with the bioparticles at 100X magnification, the needle was placed under the lens and gently pressed down onto the bioparticles, then lifted up to confirm collection of the bioparticles from the Gel-Pak film to the tungsten needle. Since each slide and sample had differing amounts of bioparticles on it, the number of bioparticles collected for each extraction depended on how much was on that particular slide.

Once the bioparticles had been collected onto the needle with the adhesive, the third microscope slide, with the LySep™ column in place, was put onto the second microscope stage. This was visualized using the macro settings, which had a total magnification of 10X, and the magnification was adjusted so that the lysis buffer in the column was in focus. The hingeless sample tube was not attached to the bottom of the column, because it was too difficult to visualize the liquid in the bottom of the column through the tube. The needle containing the ball of adhesive with the collected bioparticles was then slowly and carefully put into the column so that the adhesive on the tip of the needle was resting in the solution, without bumping the sides of the column. This was left in the solution until the adhesive was dissolved into the solution, at which point the needle could be removed from the solution. It took about 30 seconds or less for the adhesive to dissolve in the

lysis buffer. Once the adhesive had dissolved, the hingeless tube was attached to the LySep™ column. Between 3 and 7 alleged cells were collected from each slide, depending on the number of bioparticles present on the slide.

In the column, there was 220µL of lysis buffer for the PrepFiler Express™ extraction. For the PrepFiler Express BTA™ extraction there was 150µL of lysis buffer in the column.

2.4.2 Extraction, Quantification, and Amplification

In the aforementioned procedure, the one slide for each sample was used for two different extraction processes. One extraction was done using the PrepFiler Express™, which is used for bodily fluids such as during the swabbing technique or a buccal swab. The other extraction was done using PrepFiler Express BTA™ extraction, which as the acronym explains, is for bone, teeth, or adhesive. This was done in order to compare and contrast the two different extraction techniques in terms of DNA profiles obtained. These extractions were carried out as per the procedures in the PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits User Guide using the AutoMate Express™ DNA Extraction System [18].

In the PrepFiler Express™ extraction process, the total volume of the lysis solution, including DTT, was 500µL. The remainder of the master mix of lysis buffer plus DTT was added to the column/tube assembly once the adhesive was dissolved in the initial 220µL of buffer.

Similarly, in the Prepfiler Express BTA™ extraction process, the total volume of the lysis solution, which included DTT and ProK, was 230µL. The volume was adjusted with the remainder of the lysis solution once the adhesive was dissolved in the initial 150µL of lysis buffer.

A reagent blank was also extracted for each of the two extraction kits. These consisted of all the reagents used in the extraction, such as the lysis buffer, DTT, and ProK, with no DNA added.

The qPCR protocol in the user guide was followed using the Quantifiler® Human DNA Quantification kit [19].

A buccal swab belonging to the principal DNA contributor of this study, which had previously been extracted and quantified, was included along with the PCR amplification of the samples. This was in order to include a complete, known profile of the contributor, in order to make comparisons of the DNA profiles obtained later in the study. PCR was carried out as per the protocol in the user guide using the GlobalFiler™ PCR Amplification Kit [20].

The capillary electrophoresis was carried out using the Genetic Analyzer 3130 using Performance Optimized Polymer (POP4) and a 36cm capillary array. The procedure did not deviate from the protocol in the user guide [21]. There were 5 and 10-second injections done for all of the samples. The buccal swab and GlobalFiler™ DNA 007 positive amplification control were re-injected for 1 and 3-second injections. The profiles of the samples used for analysis were the 10-second injections, the only exceptions being for the buccal swab and the

GlobalFiler™ kit's DNA 007 positive amplification control, where the 1-second injections were used to eliminate noise.

2.5 Analysis of Data

Once the samples were done running on the Genetic Analyzer 3130, the data obtained was analyzed using GeneMapper®ID-X v1.4. This analysis included deleting off-ladder peaks, spikes, pull-up, dye blobs and stutter. The stochastic threshold was defined at 50 relative fluorescent units (RFUs), but there is no analytical threshold determined for the equipment in this laboratory yet.

The data was analyzed statistically using the means and standard deviations of the allele recovery percentage and allele counts. The percentage of allele recovery was calculated by dividing the number of contributor's alleles present by the total number of the contributor's alleles and multiplying by 100.

CHAPTER 3

RESULTS

3.1 Results

After quantifying the amount of DNA, the samples extracted using the PrepFiler Express™ extraction kit resulted in more DNA in pg/μL than the PrepFiler Express BTA™ extraction kit. Six of the twelve samples extracted using PrepFiler Express™ yielded a quantification result. These values ranged from 1.4-13.6pg/μL of DNA. The reagent blank yielded no results, which would indicate there was no contamination present in the extraction reagents.

Only one of the twelve samples extracted with the PrepFiler Express BTA™ extraction kit resulted in any DNA being detected and thus quantified, with a value of 4.3pg/μL of DNA. The blank in this extraction also yielded no results. All of the samples were amplified despite a lack of detection in the quantification step. Two additional negative controls and one positive control were included in the amplification step.

Regardless of the quantification results, most of the samples in each extraction type gave either a partial or mixed profile. The DNA profiles used for analysis of the data were from the 10-second injections on the capillary electrophoresis. The only exceptions were the buccal swab sample and the positive

control, where the profiles used for analysis were from the 1-second injections to reduce the noise.

The buccal swab that was amplified alongside the samples provided required information for the comparison of known alleles with the unknown samples. The female contributor had a total of 36 alleles present, and was a homozygote at eight different loci: vWA, D16S539, CSF1PO, AMEL, D8S1179, D21S11, D2S441, and D10S1248.

The samples that were extracted with PrepFiler Express™ and gave a quantification result ranged from 11 – 100% in allele recovery. The samples that did not have a value after quantification resulted 0 – 83% allele recovery.

The one sample that gave a quantification result using PrepFiler Express BTA™ resulted in 58% allele recovery. The remainder of the samples ranged from 0 – 72% allele recovery. The next highest allele recovery from samples that had no quantification result was 13%, indicating that the 72% recovery was an outlier.

Partial profiles ranged from 3 to 21 of the contributor's total 36 alleles present in the samples extracted with PrepFiler Express™ extraction. There was one full profile obtained by the samples from this extraction, which was not a mixed profile. This was sample S5R, taken from a wood substrate. There were four mixed profiles, which ranged from 1 to 9 foreign alleles present from both the clothing and writing apparatus substrates. In the case of the profile with the 9 foreign alleles (sample S9R), the foreign alleles were the major contributor. It cannot be determined if all of these alleles came from one source without additional known reference standards for comparison. This sample was from the

contributor's lab coat. There were two samples that resulted in zero alleles present. One sample was from the wood substrate (S8R) and one of these samples was from clothing (S12R; inside neck of the shirt). The reagent blank in this extraction did not obtain a quantification result, however in the DNA profile there was one allele present that did not belong to the contributor. The extraction process using the PrepFiler Express™ kit resulted in a mean 27% allele recovery ($\pm 28\%$) (Table 3.1)

All of the profiles obtained using the PrepFiler Express BTA™ extraction kit were either partial or mixed. Partial profiles, containing only the contributor's DNA, ranged from 2 to 26 of the total 36 alleles recovered. Mixed profiles had a range from 1 to 21 of the contributor's alleles recovered, but contained either 1 or 2 foreign alleles. This included one Y allele at the locus AMEL that appeared on one of the samples collected from clothing, as well as another foreign allele at locus D3S1358 (S12R). There was also a Y allele that appeared in this reagent blank, along with another foreign allele at D22S1045. Both of these samples that contained Y alleles did not have any alleles in either of the YSTR loci and no X allele alongside at the AMEL locus. There was one sample that did have an allele in one of the YSTR loci (Yindel), which was a pencil sample (S7BTA). This extraction procedure resulted in a mean 17% allele recovery ($\pm 23\%$) (Table 3.1)

The negative controls and positive control from the PCR amplification step did not show any signs of contamination. The negative controls, which were PCR reagent blanks, did not have any alleles present in the profiles. The positive

control, DNA 007, was compared to the genotype listed in Table 1 of the GlobalFiler™ User Manual [20].

The writing apparatuses (i.e. pens and pencils) resulted in a mean 25% ($\pm 22\%$) allele recovery. The wood resulted in a mean 27% ($\pm 38\%$) allele recovery. The clothing resulted in a mean 14% ($\pm 12\%$) allele recovery (Table 3.2).

Table 3.1 – Means and standard deviations for the percentage of allele recovery and the allele count out of a total 36 alleles for the extraction kits

Extraction Kit	Mean Allele Recovery %	Standard Deviation Allele Recovery %	Mean Allele Count	Standard Deviation Allele Count
PrepFiler Express™	27%	28%	10	10
PrepFiler Express BTA™	17%	23 %	6	8

Table 3.2 – Mean and standard deviations of the percentage of allele recovery and the allele count out of a total 36 alleles for the substrates

Substrate	Mean Allele Recovery %	Standard Deviation Allele Recovery %	Mean Allele Count	Standard Deviation Allele Count
Writing Utensil	25 %	22%	9	8
Wood	27%	38%	10	14
Clothing	13%	12%	5	4

CHAPTER 4

DISCUSSION

4.1 Discussion

4.1.1 Tapelifting Aluminum Stubs

The selection of which aluminum stub would be most useful required several different trials comprising many different factors. The Gel-Pak film was used, as it was the tapelift that was used in the Farash et al. study [4]. A poster from the same research group was found that evaluated the use of three different adhesives to collect epithelial cells, Gel-Pak film was one of the adhesives that was tested [22]. There were a total of nineteen trials involved in the selection of the aluminum stub. The initial trials (Trials 1 – 12) tested the adhesion of the Gel-Pak film to the black carbon adhesive on the TriTech stubs. Trials 13 to 16 involved both types of stubs. The last three trials used only the Ted Pella stubs, and were used to test the best double-sided tape. Ultimately, the black carbon adhesive adhered the Gel-Pak film too well, making it next to impossible to remove the Gel-Pak and place it onto a microscope slide. The adhesive would become deformed and no longer sticky, while also retaining a large amount of the carbon adhesive with it. The carbon that transferred to the tape interfered with the visualization of bioparticles, when observed under the microscope. Instead, the Ted Pella stubs were tested in order to determine if the Gel-Pak would come off the aluminum stub more easily without a secondary adhesive, such as the carbon adhesive. These trials were also unsuccessful. During the testing of the double-

sided tape, to see if these adhesives would allow for easier removal of the Gel-Pak, the idea to leave the white backing on the Gel-Pak film was formed.

Once the idea to keep the backing on the Gel-Pak had come to fruition, the double-sided tape that would adhere the white backing of the Gel-Pak the best had to be chosen. The Scotch® Wallsaver Removable Poster Tape did not even hold the Gel-Pak in place before any disturbance to it. This option was disregarded immediately. The 3M Scotch® Mounting Tape included a layer of foam, which ultimately included no benefits to the collection of DNA. This tape was also difficult to measure and cut due to its thickness, which led to this tape not being chosen. The double-sided tape that was selected was chosen for its convenience, thickness and ability to hold the Gel-Pak in place. However, once the sampling began, it became apparent that this tape was not strong enough to hold the Gel-Pak in place for sampling all types of substrates. The Gel-Pak would remain stuck to the substrate and come off the stub in the case of metal (e.g. light switch panel, pop can, laptops, door handles), beer bottles, and magazines. This is a limitation of this double-sided tape, which was a generic brand obtained from Dollarama. The TriTech stubs were tested again at this point, to see if the carbon adhesive would adhere to the backing of the Gel-Pak film well, but it did not. In further studies, it would be wise to choose another type of double-sided tape that would adhere to the backing of the Gel-Pak film more strong than the Gel-Pak film adheres to substrates. However, the idea to leave the white backing on provided many advantages to this procedure, including no interference with the adhesive on the back of the Gel-Pak.

The time it took to prepare 30 stubs was about 90 minutes. Preparation included inserting the aluminum pin into the mount, measuring and cutting the double-sided tape, and finally, the measuring, cutting and placement of the Gel-Pak film onto the stub. This gives approximately a 3-minute in-lab preparation time for each stub.

4.1.2 Collection of DNA

There was DNA collected from three different types of substrates, each with its own advantages and disadvantages. It has been shown that porous substrates are better for the collection of touch DNA, since the DNA is able to diffuse within the porous substrate, whereas with non-porous materials, the DNA and bioparticles are subject to being wiped off or exposed to environmental factors [11].

The substrate that gave the highest mean yield of DNA was the wood, followed by the writing utensils, and then the clothing. These results were interesting because during the micromanipulation process, the wood samples were the most difficult to analyze, followed by the clothing. However, when the standard deviations of these means are considered, the wood samples showed the most variability of all the substrates. Wood has been shown to give good results in other studies using tapelifts through direct extraction of the tapelift [15]. The Gel-Pak film lifted wood debris and dirt, along with bioparticles, which made it difficult to distinguish between particles of wood debris and bioparticles. The collection method for the wood samples had become a “blind-collection.” Yet, the only full profile that was obtained in this study was from a wood sample.

However, there were two wood samples (S5R and S8BTA) that did not have any of the major contributor's DNA present. The clothing samples were difficult to manipulate under the microscope as well. The bioparticles were easy enough to distinguish, but during collection with the tungsten needle fibres and hairs were also collected on the solder tape adhesive ball. This provided less surface area for the bioparticles to be collected on the adhesive.

The PrepFiler Express™ extraction in general gave more favourable results in terms of percentage of allele recovery as compared to the PrepFiler Express BTA™ extraction. The same slides were used for both extractions. An identical number of bioparticles was collected for each sample between extractions, though the number of bioparticles collected differed between each slide. The bioparticles for the PrepFiler Express™ extraction were collected first. There is the possibility that the better cells that contained nuclei were collected during the first half of the collection process.

4.1.3 Modification of the Protocol

Farash et al. provided a comprehensive outline for their micromanipulation technique, which was followed closely in this study, including many of the same materials [4]. However, their protocol was outlined in order to produce the best allele recovery possible using this micromanipulation technique, not so that it followed the standard operating procedure in a forensic laboratory [7].

One of the modifications to the protocol was the way the DNA was collected. While the use of the Gel-Pak film in this study was the same, the

collection apparatus on which the adhesive was adhered to was different. Farash et al. put the Gel-Pak directly on the microscope slide that was to be used for the analysis of bioparticles under the microscope [4]. There are several disadvantages to using a microscope slide as the apparatus for collection. One disadvantage is the delicate nature of the microscope slide, which could break during collection, thus resulting in the loss of sample. On the other hand, the size of the microscope slide proved to be both an advantage and disadvantage. The size of the slide does not allow for the collection of touch DNA in hard-to-reach places, nor does it allow for easy manipulation. The size does however prove to be an advantage in the fact that the Gel-Pak film square that is used can be of a larger surface area, thus capable of collecting more touch DNA. Nevertheless, there are several advantages to the use of the aluminum stub as a modified tapelifting technique. The aluminum stubs, which are often used for GSR evidence or SEM analysis, are readily available and familiar in most forensic laboratories. They provide distance between the collector and the sample during the collection process, which provides just one of the many barriers put in place to avoid contamination. The size of the stub also allows for easy handling and manipulation during the collection process. These advantages in the collection process have been acknowledged in other studies that involve the use of aluminum stubs [5, 13]. The plastic vial of the stub provides additional protection for avoiding contamination. The plastic vial also allows for the stub to be reclosed post-collection.

After the Gel-Pak was on the microscope slide, Farash et al. suggested the use of an optional Trypan Blue stain for the visualization of cells [4]. Trypan Blue

stains dead cells, which are permeable to the dye. This was attempted for a few of the trial runs at the beginning, when the micromanipulation technique was just being practiced, but the option to not use the dye was chosen for personal preference.

Another modification that was made to the protocol was the inclusion of the extraction and quantification steps during DNA analysis. These steps were omitted from the Farash et al. protocol and their reasoning was in order to not waste any of the already minute amounts of DNA from step to step [4]. While the allele recovery may have been good for their study, it is not an accurate representation of the results in a forensic laboratory if this were a technique to be done in casework. Though larger sample numbers would have been better in this project, the research shows that full profiles can be obtained through this method, including extraction and quantification using as little as four cells.

Following this modification, this study included two different types of extractions. The purpose of this was to compare the PrepFiler Express™ extraction kit and the PrepFiler Express BTA™ extraction kit. The hypothesis was that because the PrepFiler Express BTA™ kit is intended for adhesives, there was the possibility that this extraction would work better because the DNA was entering the lysis buffer by way of a water-soluble adhesive. The results did not indicate that the PrepFiler Express BTA™ extraction was better; in fact PrepFiler Express™ extraction resulted in better allele recovery.

Other modifications that were made included the use of a comparison microscope with a maximum magnification of 400X, not an expensive model of

stereomicroscope as the paper suggested to use. This involved less room to manipulate the tungsten needle between the slide and the lens, while also working at lower magnifications. While this may have been a minor drawback, it is a realistic representation of the equipment that most forensic laboratories would have access to and did not make the technique impossible. The comparison microscope also proved to be an advantage in the end, because it allowed for minimal moving and switching of slides from underneath the lens. This also results in less handling of the microscope slide, which could result in contamination.

4.1.4 qPCR Results Compared to Profile Output

Between both extractions, there were only seven samples that yielded a quantifiable amount of DNA. Since there were a few samples that had quantification results, the entire sample lot was put through PCR and capillary electrophoresis, because it is not uncommon for samples to be undetected in qPCR but give a DNA profile output [23]. Quantifiler™ is an appropriate kit for the prediction of whether an STR profile will be obtained. After amplification and injection on the capillary electrophoresis, there were only two samples that resulted in zero alleles detected. Sometimes the kit is correct and there is no DNA present, such as in the two samples of this study. However, there were 15 other samples that gave partial profiles despite no quantification value. The software for the kit is able to extrapolate beyond the range of the standard curve (0.023-

50ng/ μ L) to roughly estimate the quantification value. However, these estimates can change from run-to-run [23]

4.1.5 Injection Times

The injection times were the same for all of the samples at 10-seconds, but not for the buccal swab or the positive control. The samples required longer injection times, because they had lower amounts of DNA. Ideally, the peak heights, measured in relative fluorescent units (RFU), would increase with injection time. The buccal swab was about 66ng/ μ L and the positive control DNA 007 concentration is 0.1ng/ μ L. However, these had much more DNA and did not require injection times that were as long. By reducing the injection times for these two samples, the noise was also reduced which allowed for a much cleaner DNA profile, which allowed for easier interpretation.

4.1.6 Limitations of the Study

A major limitation of this study is the low number of samples, which does not allow for many statistically significant conclusions to be drawn. The low sample number resulted in high variability, which is shown through the large standard deviations of both the extractions and the substrates. Another limitation was the poor choice in double-sided tape, which ultimately led to fewer types of substrates that could be tested. As well, the in-lab preparation of the aluminum stubs required about 3 minutes per stub. However, the substrates that were tested encompassed forensically relevant items. The micromanipulation technique

required practice to master the meticulous manipulation of the tungsten needle under the microscope. Even once the technique was well practiced, the procedure was time-consuming to ensure precision.

CHAPTER 5

CONCLUSIONS

5.1 Summation

In summation, the micromanipulation technique and the stubbing method that were proposed in this study worked well in tandem and independently. The tapelifting method provides many advantages over the common swabbing method. The stubbing method contributes and adds to the advantages already in place from the basic tapelifting techniques. The aluminum stub is a common and familiar tool in forensics, as it is the method of collection for gunshot residue evidence as well as in scanning electron microscopy. The stub is easy and comfortable to hold during collection of evidence. The plastic vial prevents contamination by covering the aluminum pinhead and subsequently the tapelift on the pinhead. The assembly of the stubs, for the purpose of acting as a tapelift, is simple but does require time. However, the advantages outweigh the disadvantages.

The micromanipulation technique that was outlined by Farash et al. proved to be an adequate method for the collection of touch DNA. Even with the modifications to the method of analysis, the protocol still resulted in output. Many partial profiles were produced and one full DNA profile using seven bioparticles or less.

The two extraction methods that were compared, PrepFiler Express™ and PrepFiler Express BTA™, both resulted in mostly partial profiles for the samples extracted with the kits. The samples extracted with PrepFiler Express™ DNA

extraction kit resulted in a better allele recovery than those with PrepFiler Express BTA™ DNA extraction.

5.2 Future Research and Recommendations

This study has potential to act as a preliminary study for future research. This experiment could be carried out with more samples, which would allow for more definitive conclusions to be made about the efficacy of the techniques. This could either incorporate new substrates or focus on the substrates that were used in this study. It would be wise to choose a stronger double-sided tape to hold the Gel-Pak backing onto the stub, which would only improve an already functional method. This method of analysis should also be attempted using different donors, as well as donors that are not the same person collecting and analyzing the samples.

APPENDIX I

Raw Data

A.1 – Extraction, Quantification, and Allele Recovery using PrepFiler Express™ DNA extraction kit

Sample	Source of DNA	# of cells collected	Ng/uL	Pg/ul	# of contributor's alleles	# of foreign alleles present	AMEL	% of Allele Recovery
S1 R	Pen (Papermate)	7	0.001596	1.596	11	3	X	30.56%
S2 R	Wood (Dirty; red paint)	5	-	-	3	0	X	83.33%
S3 R	Back neck of coat	6	0.001424	1.424	14	1	X	38.89%
S4 R	Pen (Papermate)	6	0.001408	1.408	21	0	X	58.33%
S5 R	Wood	4	0.013598	13.598	36	0	X	100%
S6 R	Scarf	6	-	-	7	2	X	19.44%
S7 R	Pencil (Staples)	5	0.001524	1.524	6	0	-	16.67%
S8 R	Wood	4	-	-	0	0	-	0%
S9 R	Lab coat	3	0.00279	2.79	4	9	X	11.11%
S10 R	Pen (Papermate)	6	-	-	6	0	-	16.67%
S11 R	Wood	3	-	-	8	0	X	22.22%
S12 R	Inside neck of shirt	7	-	-	0	0	-	0%
Blank R	Reagents		-	-	0	1	-	0%

A.2 – Extraction, Quantification, and Allele Recovery using PrepFiler Express BTA™ DNA extraction kit

Sample	Source of DNA	# of cells collected	Ng/uL	Pg/uL	# of contributor's alleles	# of foreign alleles	AMEL	% allele recovery
S1 BTA	Pen (Papermate)	7	-	-	3	1	-	8.33%
S2 BTA	Wood (Dirty; red paint)	5	-	-	3	1	-	8.33%
S3 BTA	Back neck of coat	6	-	-	3	1	-	8.33%
S4 BTA	Pen (Papermate)	6	-	-	1	1	-	2.78%
S5 BTA	Wood	5	-	-	0	1	-	0%
S6 BTA	Scarf	7	-	-	4	0	X	11.11%
S7 BTA	Pencil (Staples)	6	0.004313	4.313	21	2	-	58.33%
S8 BTA	Wood	4	-	-	1	1	-	2.78%
S9 BTA	Lab coat	3	-	-	2	0	-	5.56%
S10 BTA	Pen (Papermate)	6	-	-	4	2	X	11.11%
S11 BTA	Wood	3	-	-	26	0	X	72.22%
S12 BTA	Inside neck of shirt	7	-	-	5	2	Y	13.89%
Blank BTA	Reagents		-	-	0	2	Y	-

A.3 – Foreign alleles present in the samples

Sample	Locus	Allele	Size	Height	Allele 2*	Size	Height
S1BTA	D8S1179	12	142.51	89			
S1R†	D3S1358	15	121.23	54			
	SE33	34	426.73	73	35	430.69	61
S2BTA	D13S317	15	239.06	67			
S3BTA	D13S317	8	210.71	62			
S3R‡	D13S317	12	227.13	71			
S4BTA	CSF1PO	12	306.57	69			
S5BTA	vWA	24	209.42	58			
S6R‡	D8S1179	6	118.35	51			
	D13S317	12	226.99	63			
S7BTA	vWA	19	189.06	66			
	Y indel	2	86.38	86			
S8BTA	TH01	6	186.88	89			
S9R‡	D3S1358	16	125.41	94	18	133.34	81
	D8S1179	9	130.42	56			
	D21S11	29	203.42	142			
	D2S441	14	101.23	106			
	D5S818	11	154.94	147	12	159.03	152
	D13S317	12	227.02	158			
	D10S1248	16	117.85	133			
S10BTA	D18S51	15	293.51	72			
	SE33	18	362.23	78			
S12BTA†	D3S1358	15	121.09	81			
	AMEL	Y	104.76	95			
Blank	AMEL	Y	104.74	88			
BTA	D22S1045	16	112.35	50			
Blank R	CSF1PO	11	302.63	56			

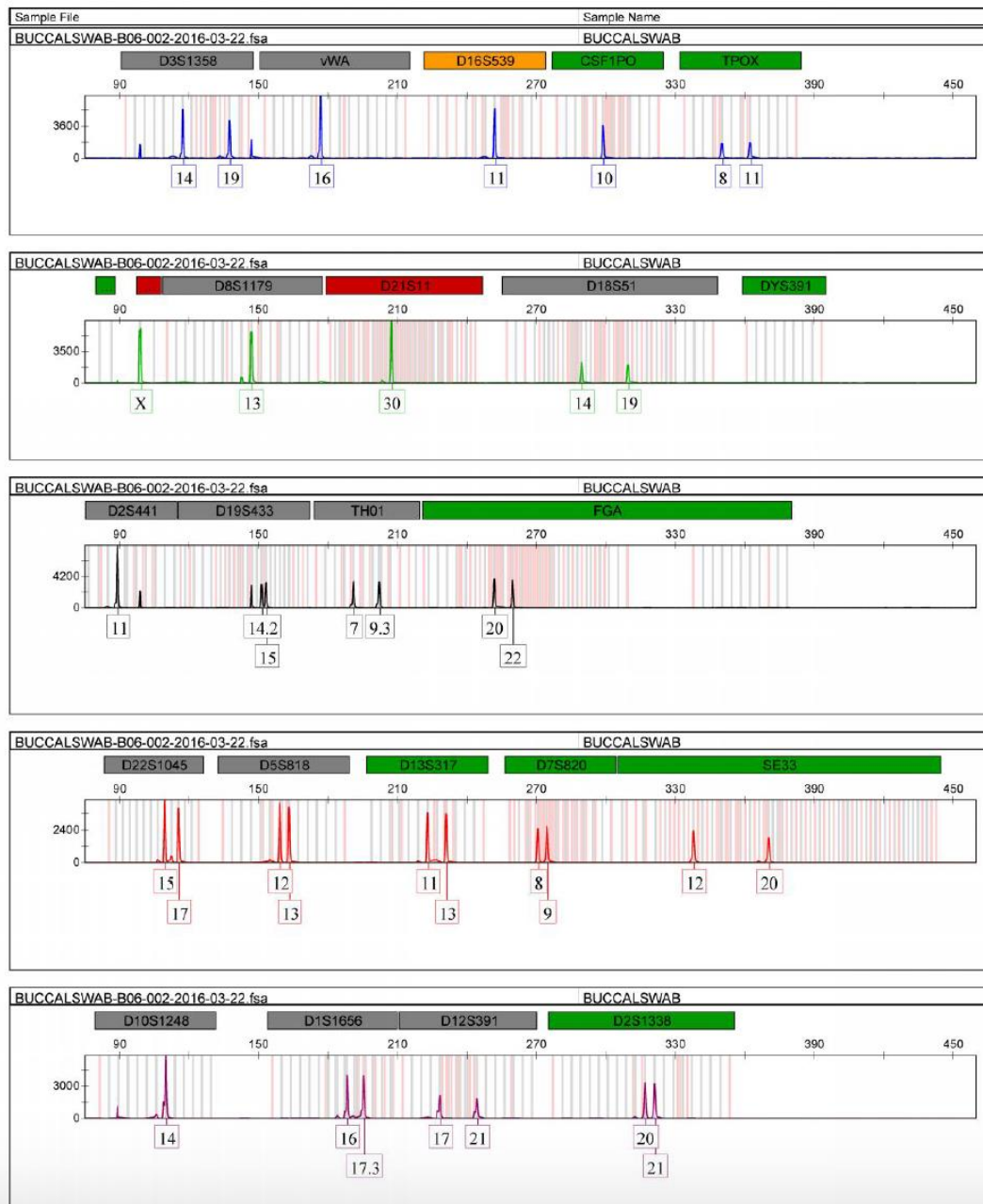
* ‘Allele 2’ refers to the fact that there are multiple foreign alleles present at the same locus

† These samples have the same allele call (15) at locus D3S1358

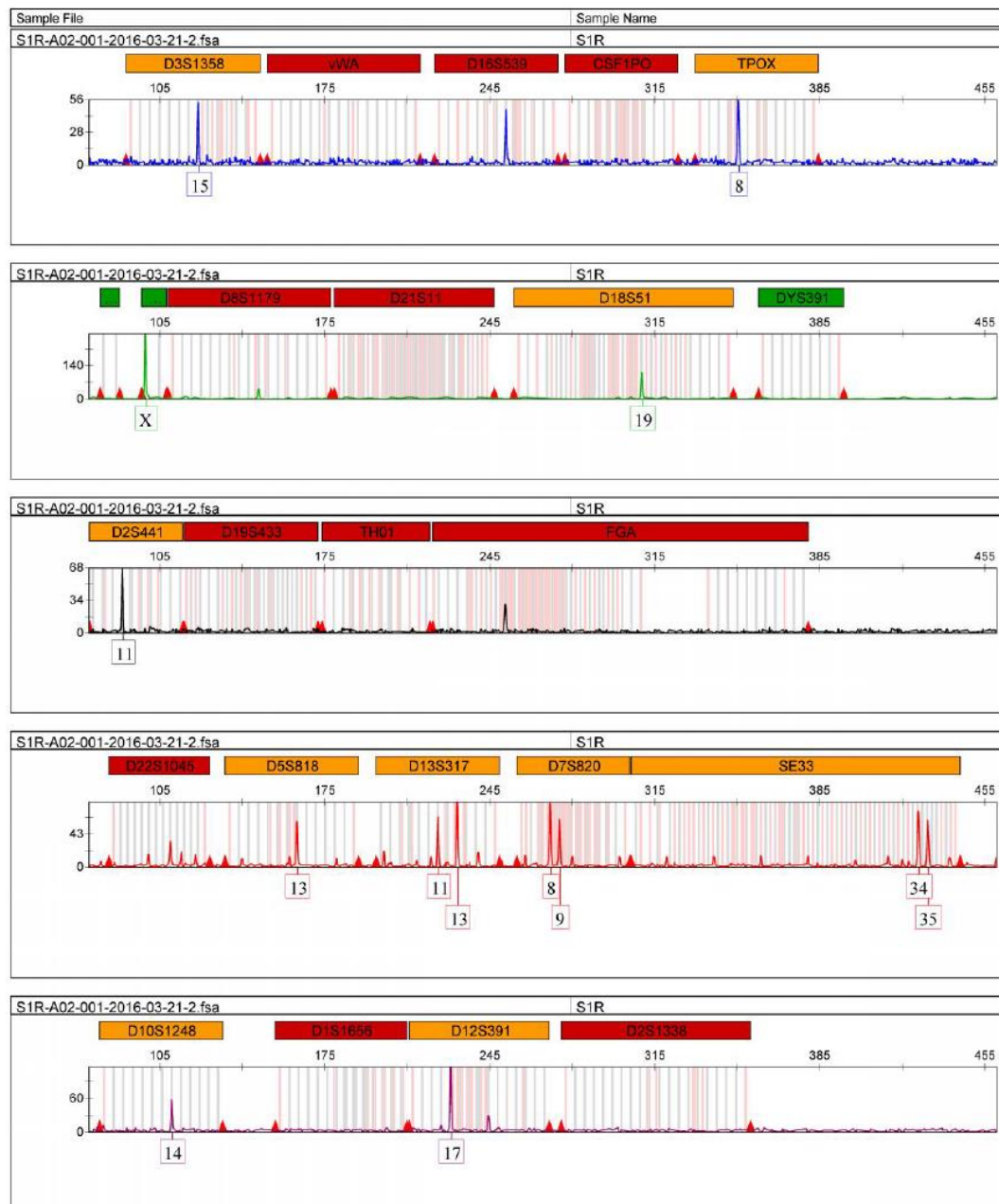
‡ These samples have the same allele call (12) at locus D13S317

APPENDIX II

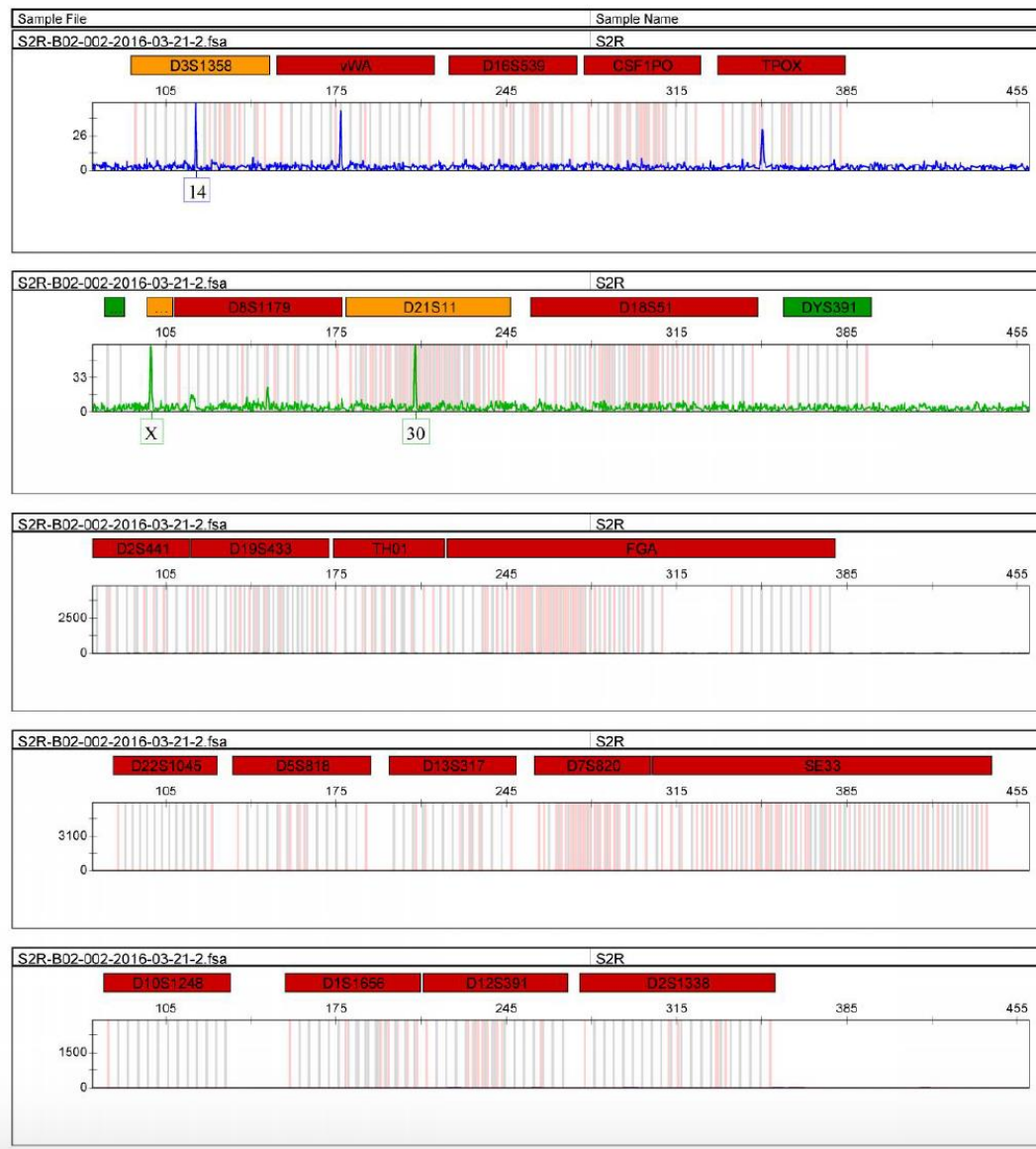
DNA Profiles



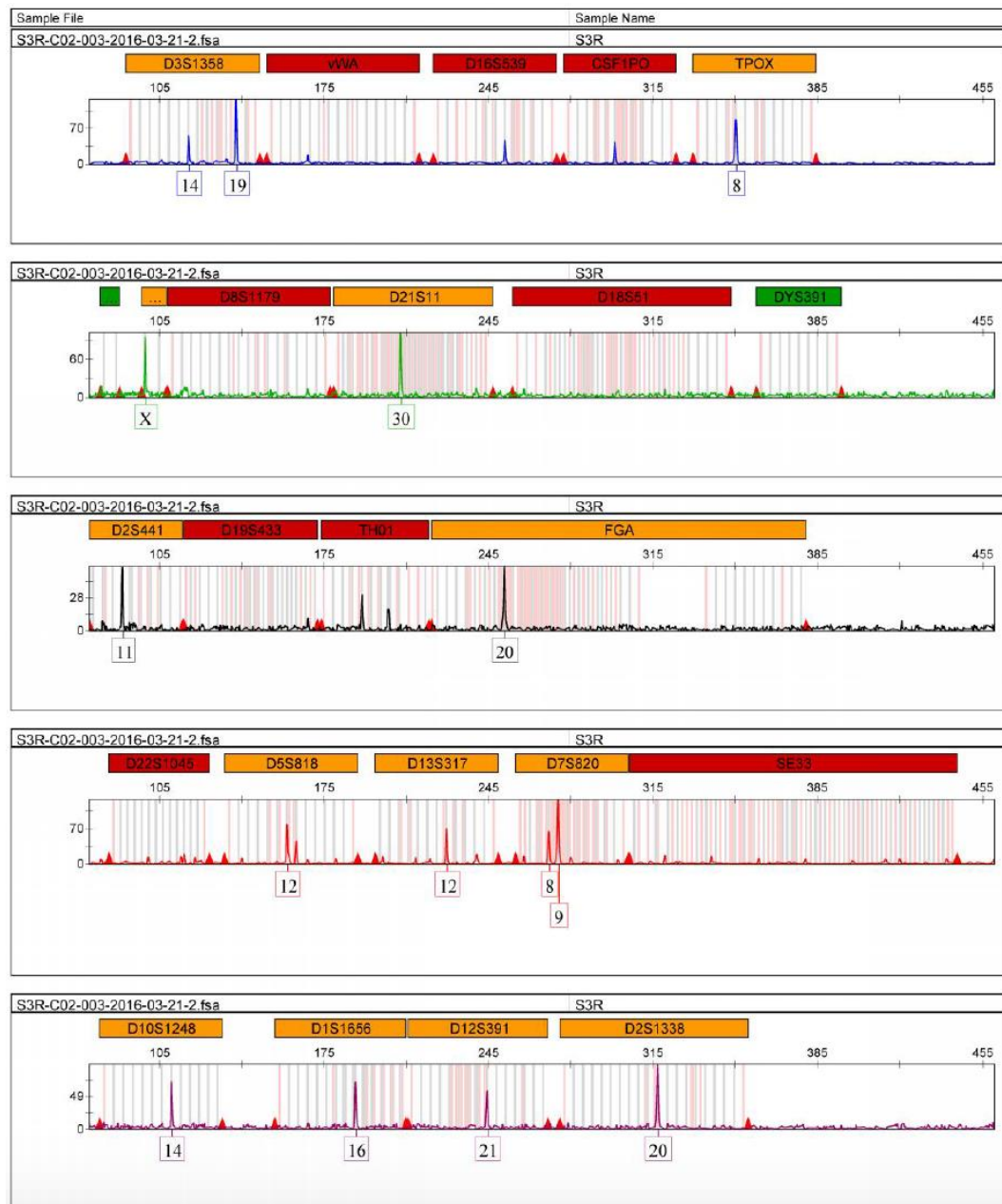
Buccal Swab
 PrepFiler Express™
 1-second injection



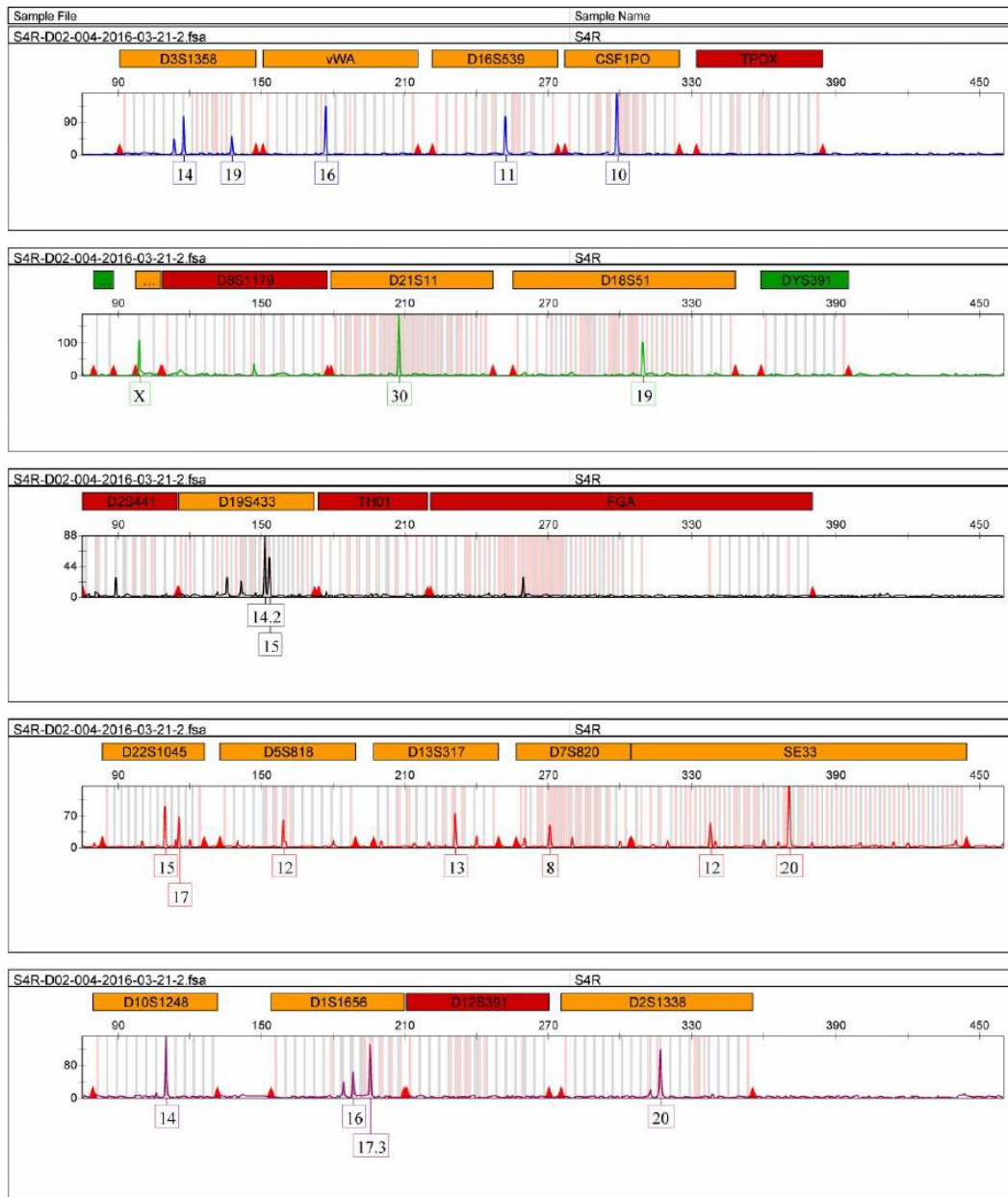
Sample 1
 Pen (Papermate)
 PrepFiler Express™
 10-second injection



Sample 2
 Wood (Dirty, red paint)
 PrepFiler Express™
 10-second injection



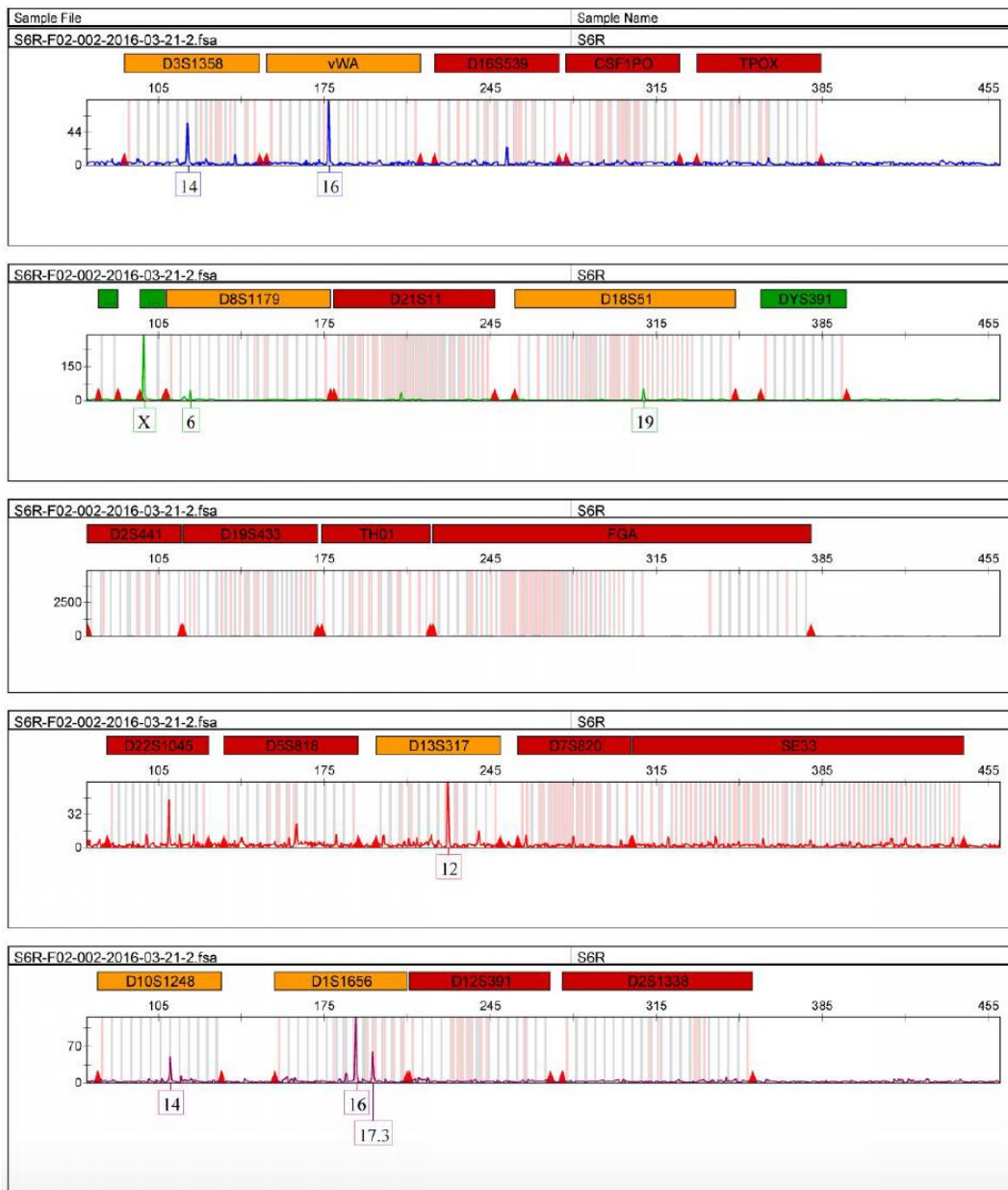
Sample 3
 Back neck of coat
 PrepFiler Express™
 10-second injection



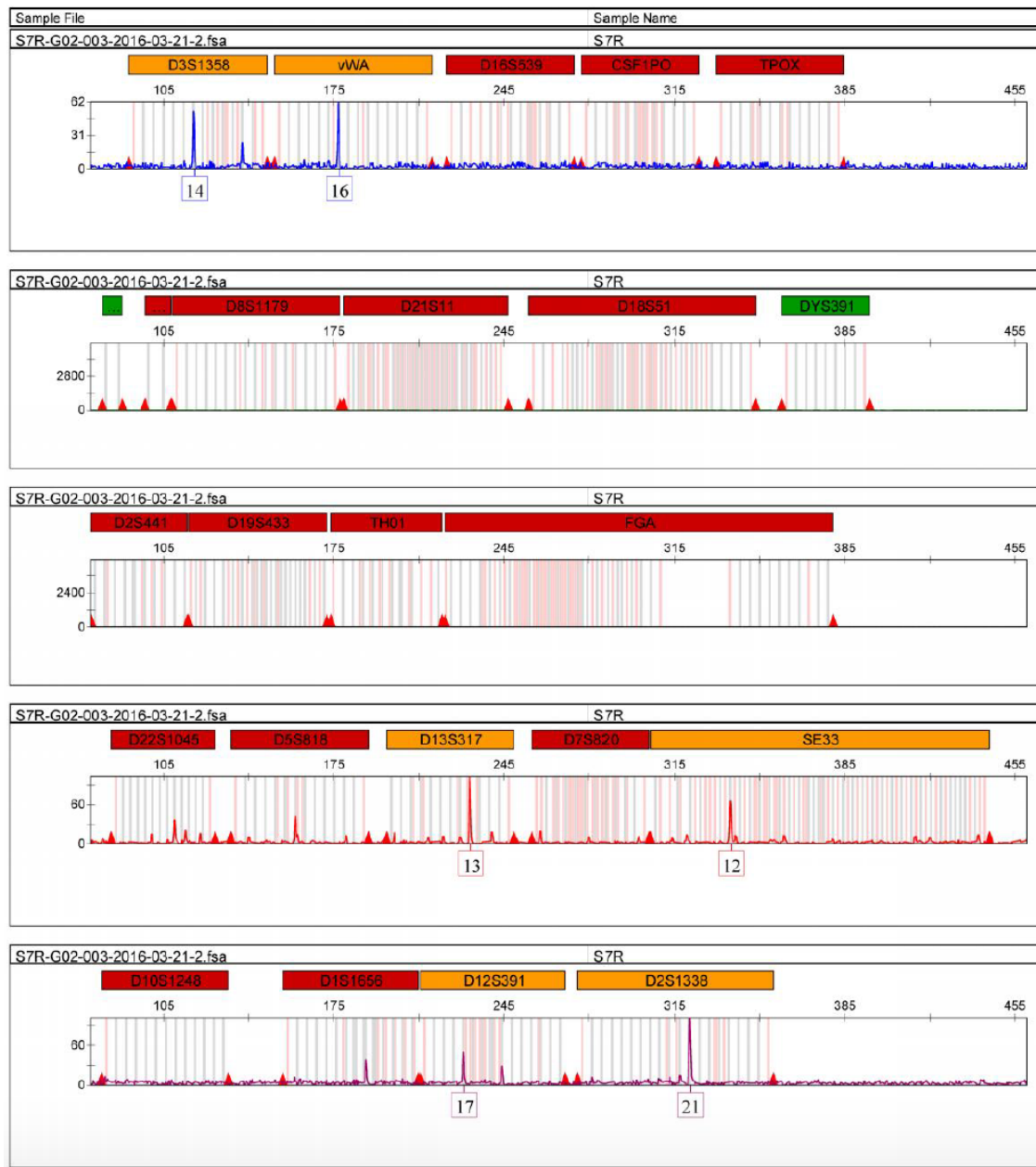
Sample 4
 Pen (Papermate)
 PrepFiler Express™
 10-second injection



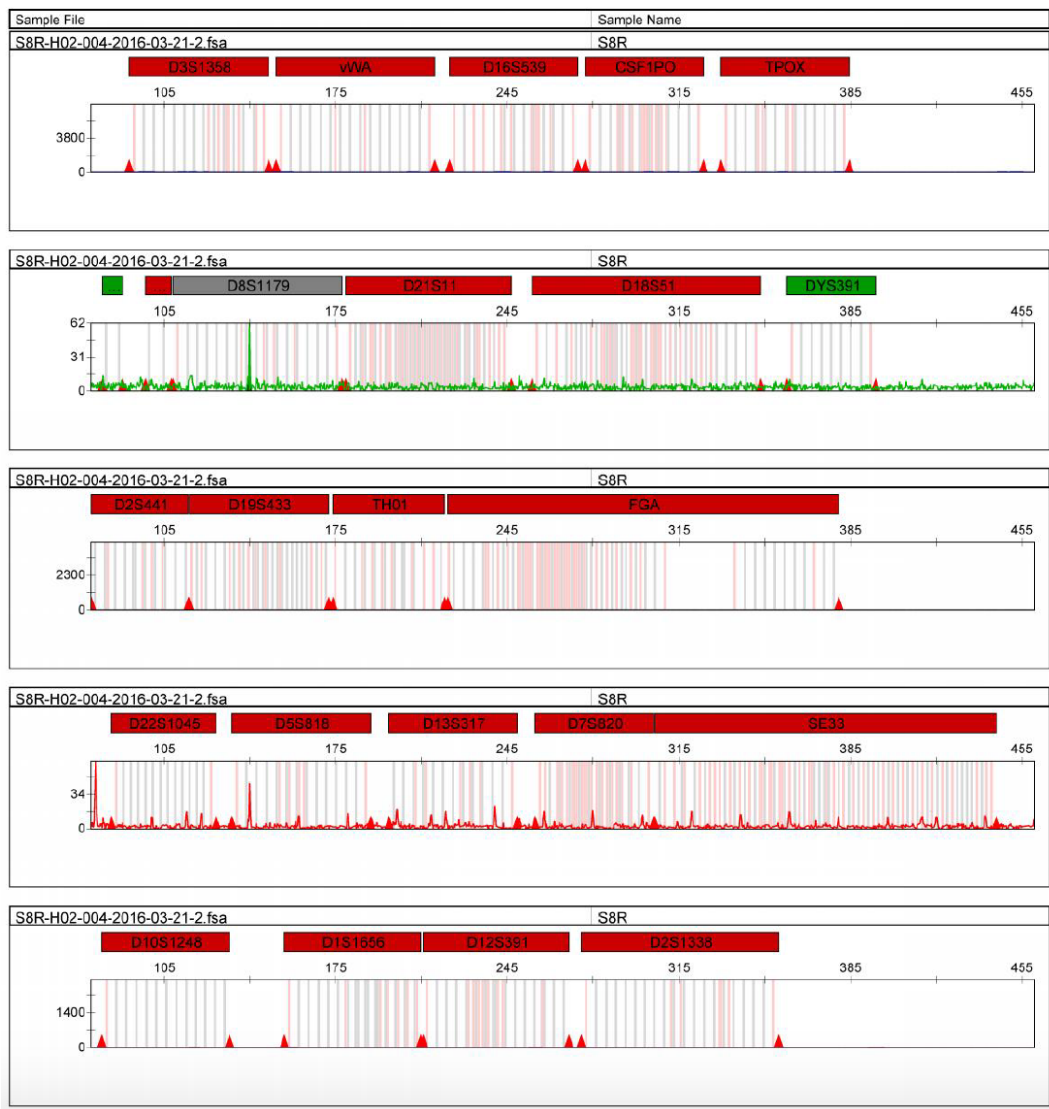
Sample 5
 Wood (Clean)
 PrepFiler Express™
 10-second injection



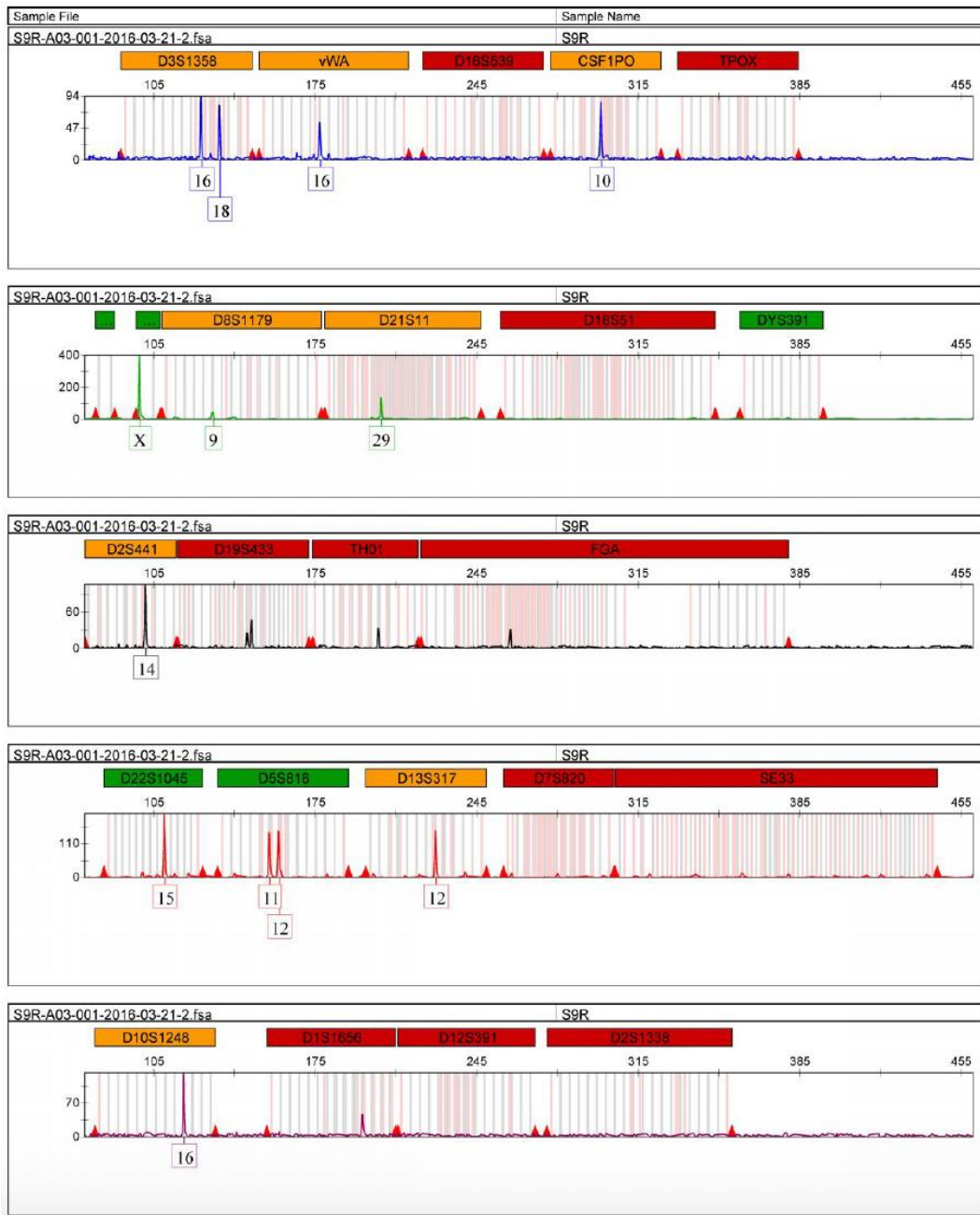
Sample 6
 Scarf
 PrepFiler Express™
 10-second injection



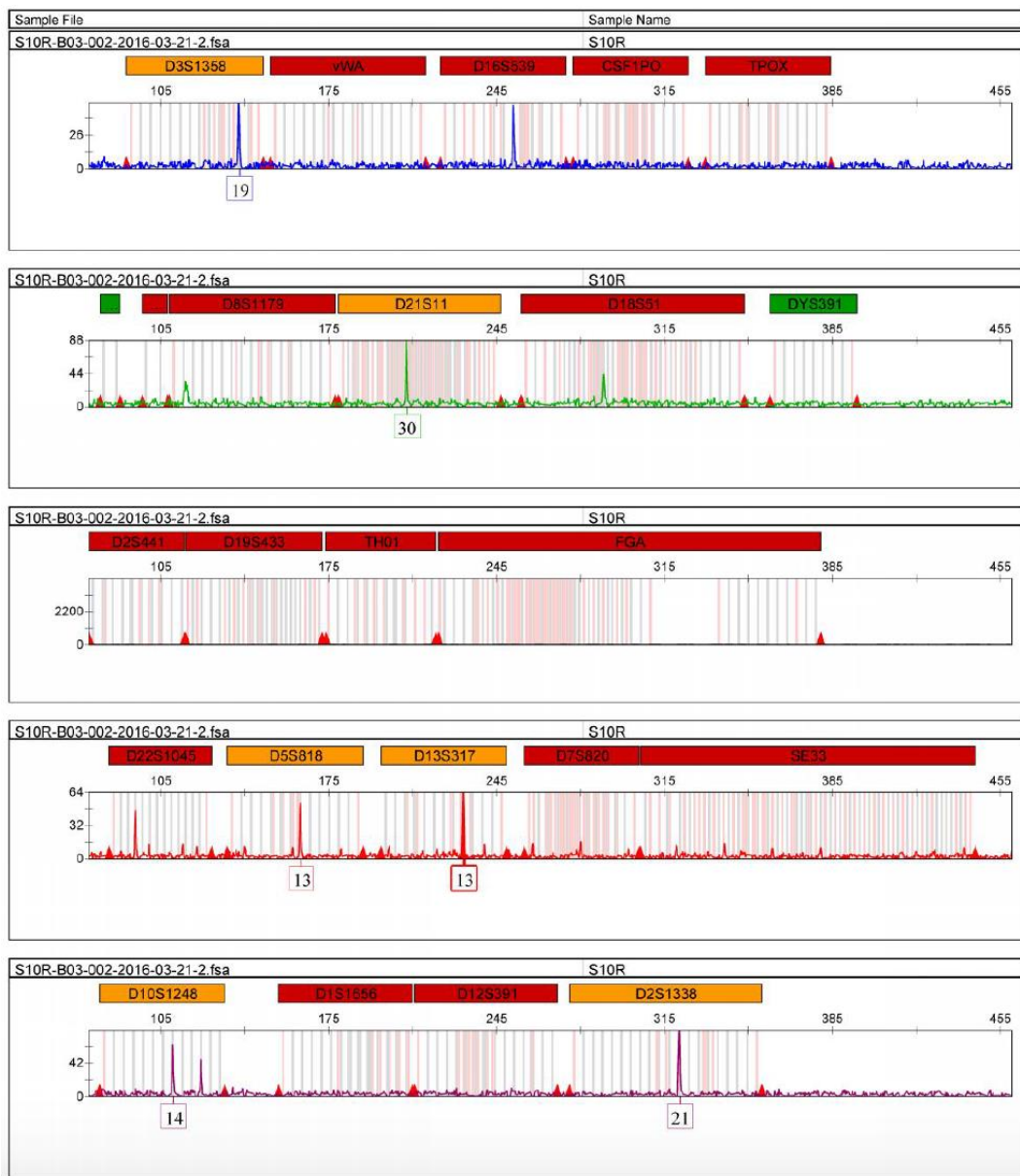
Sample 7
Pencil (Simply Wood)
PrepFiler Express™
10-second injectio



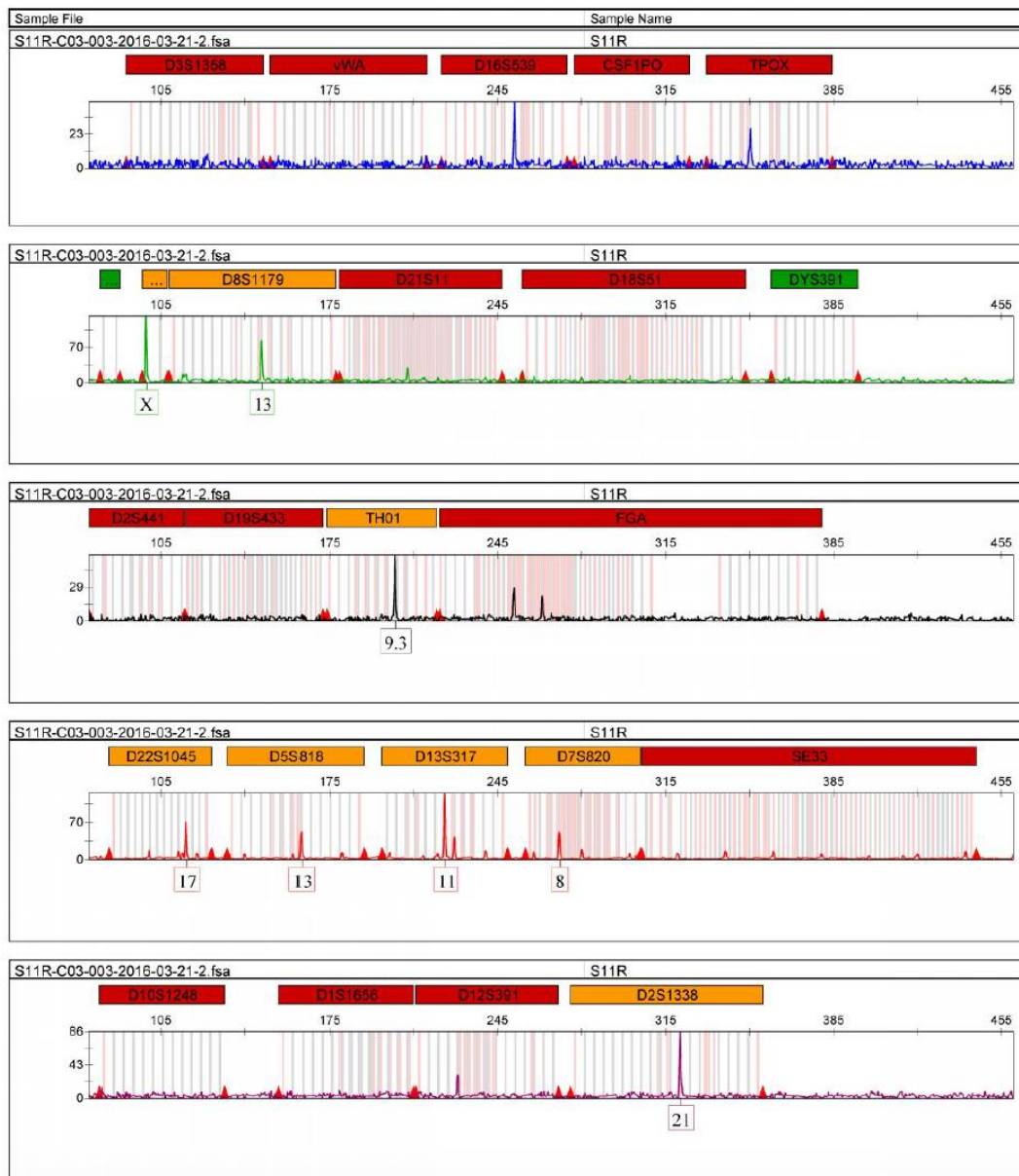
Sample 8
 Wood (Dirty, brown paint)
 PrepFiler Express™
 10-second injection



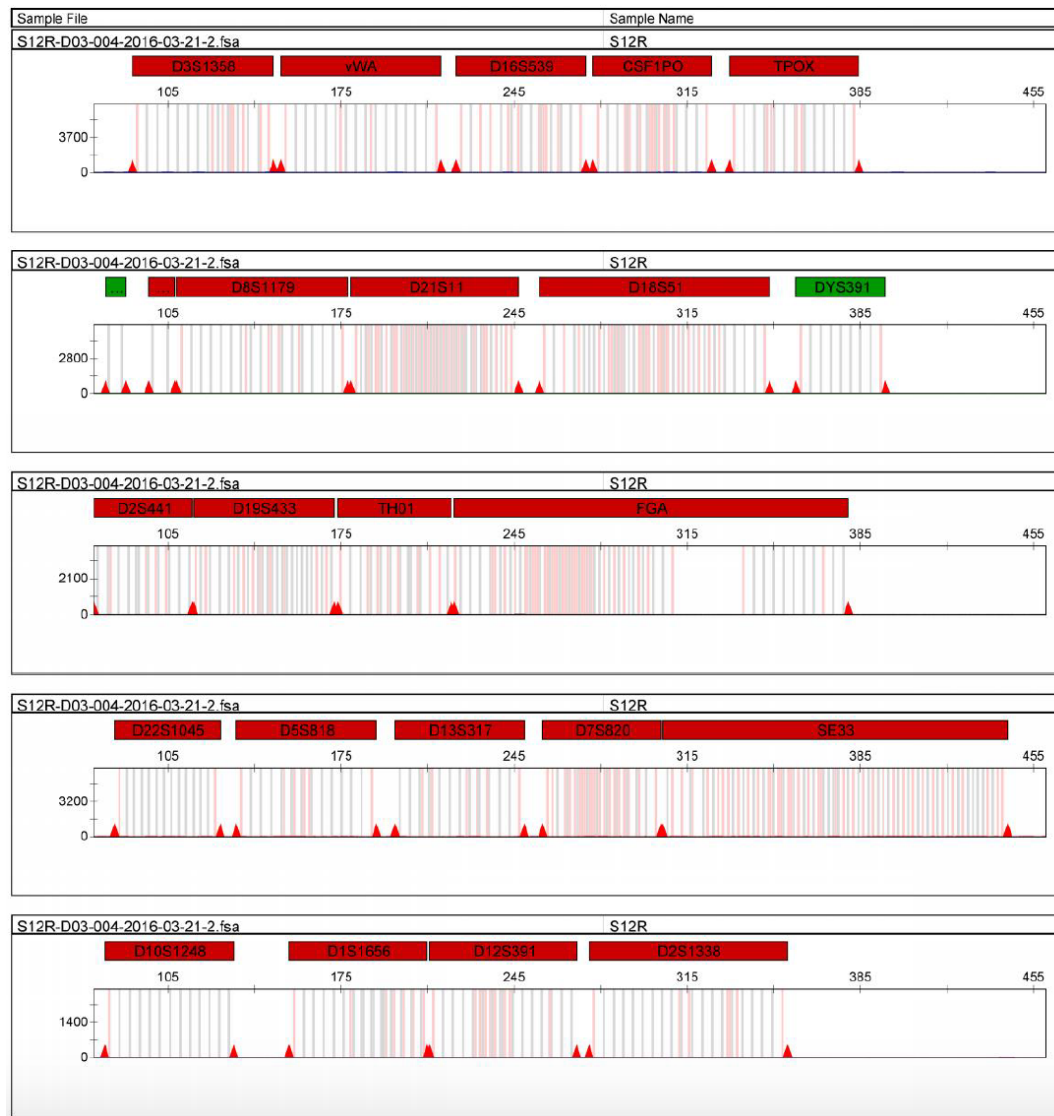
Sample 9
 Lab coat
 PrepFiler Express™
 10-second injection



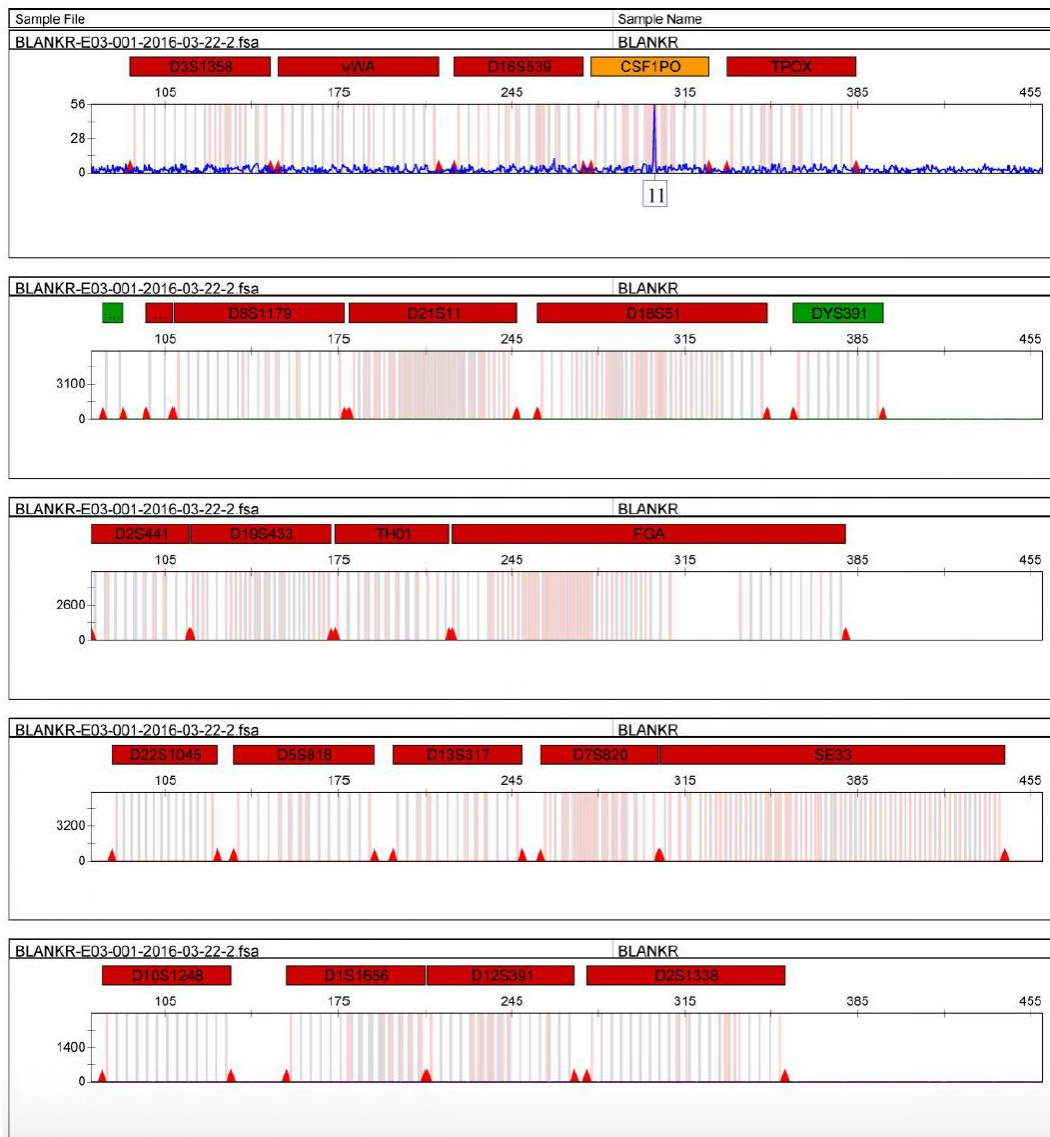
Sample 10
 Pen (Papermate)
 PrepFiler Express™
 10-second injection



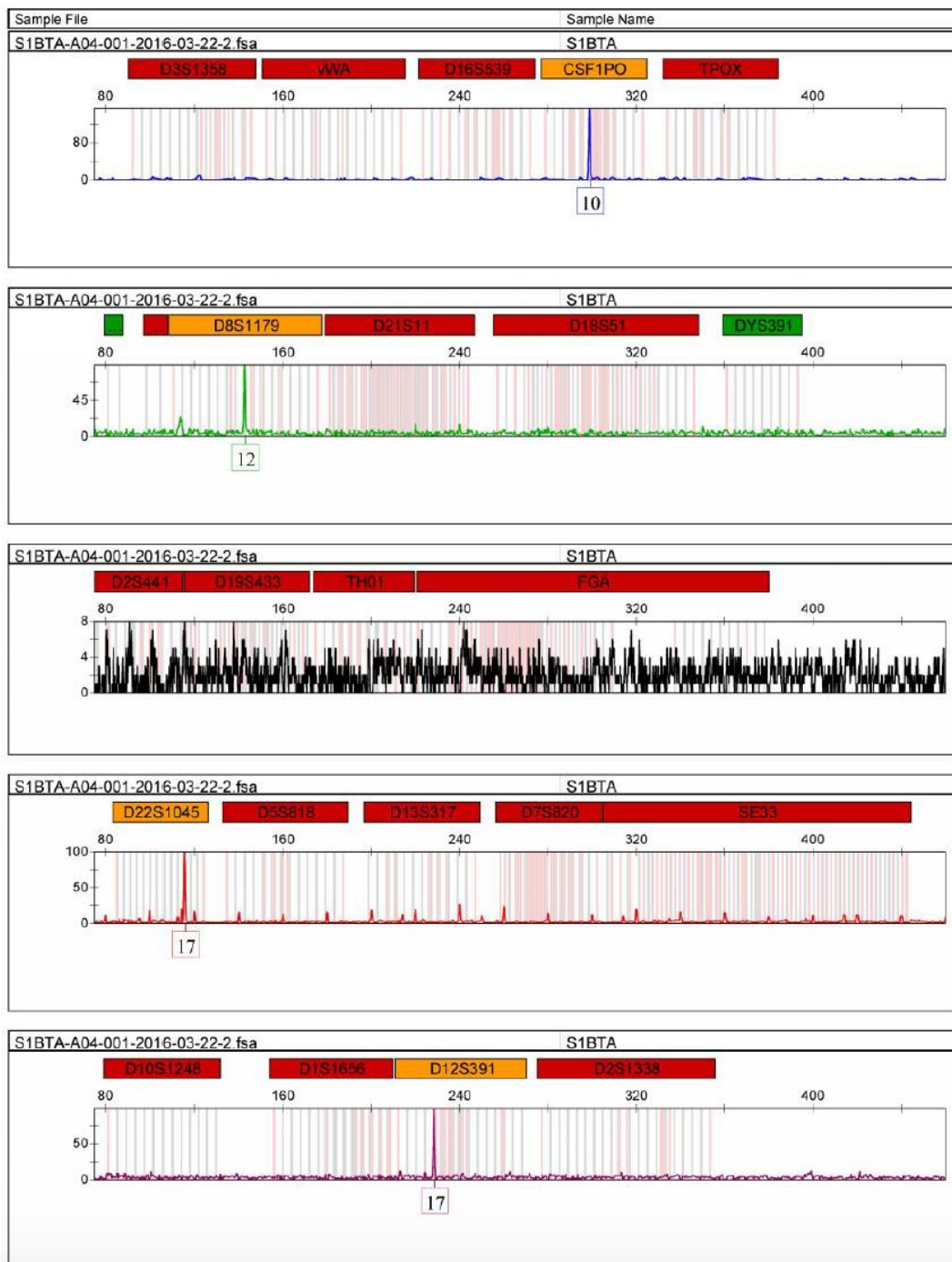
Sample 11
 Wood (Clean)
 PrepFiler Express™
 10-second injection



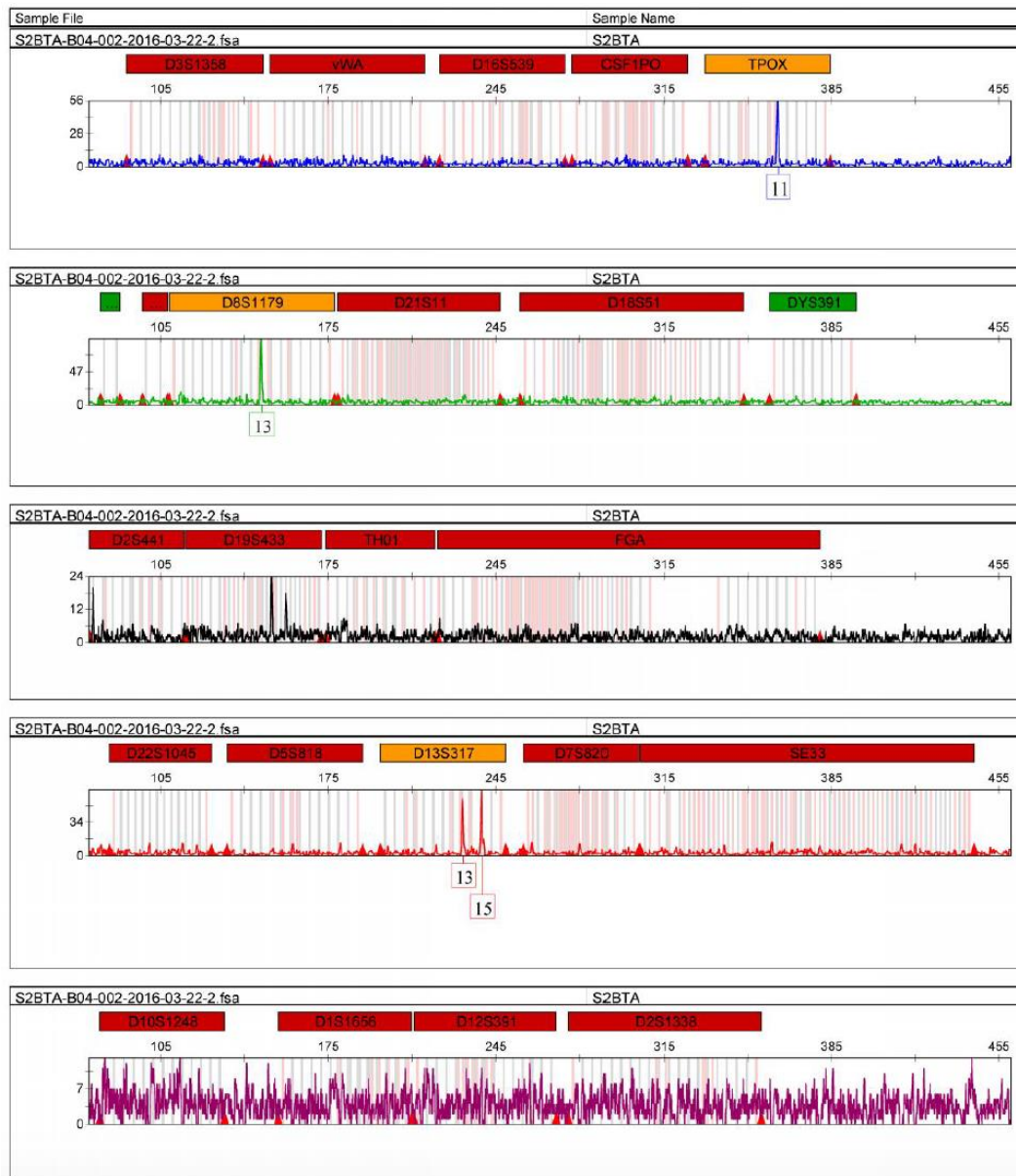
Sample 12
 Inside neck of shirt
 PrepFiler Express™
 10-second injection



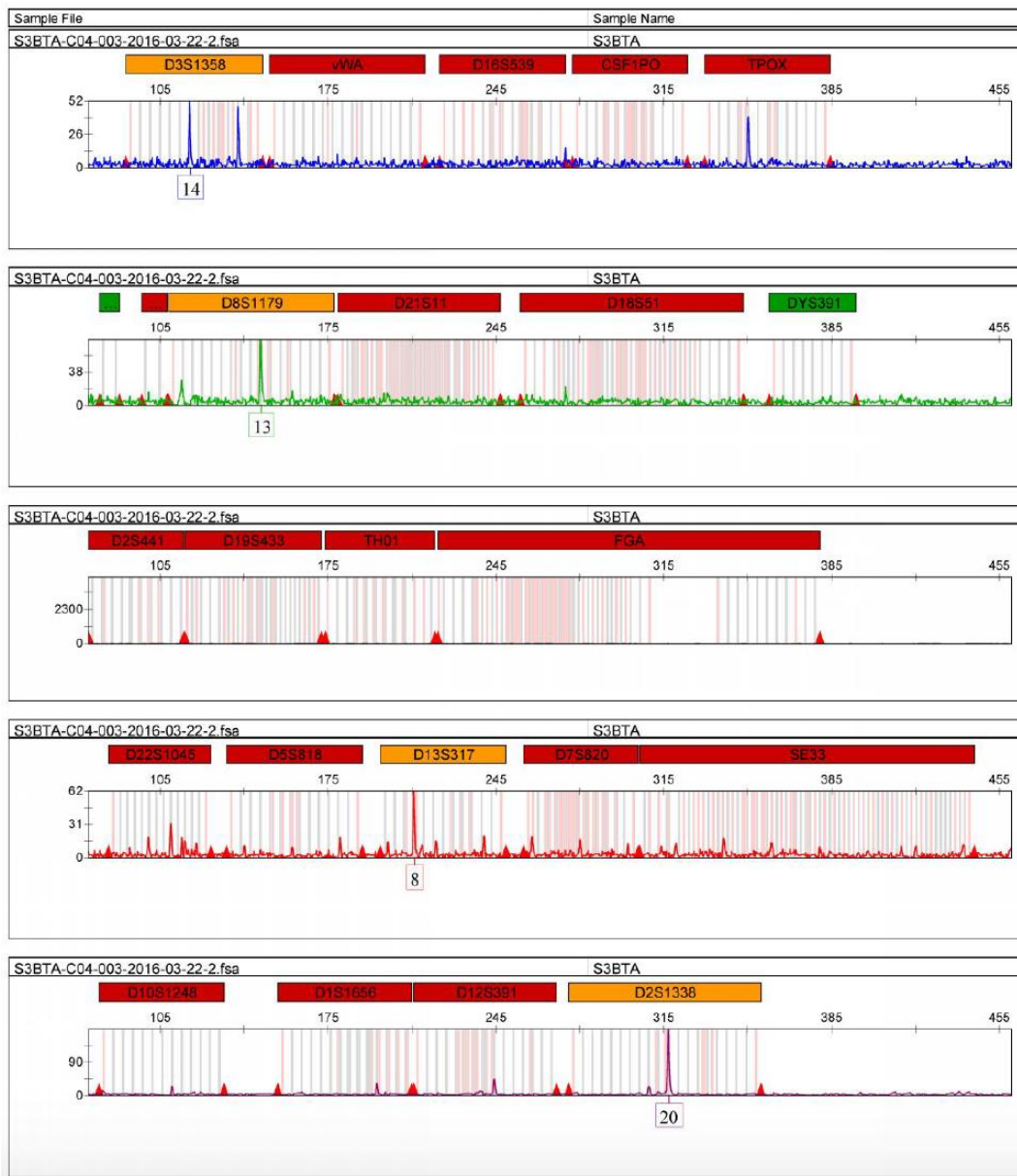
Reagent Blank
 PrepFiler Express™
 10-second injection



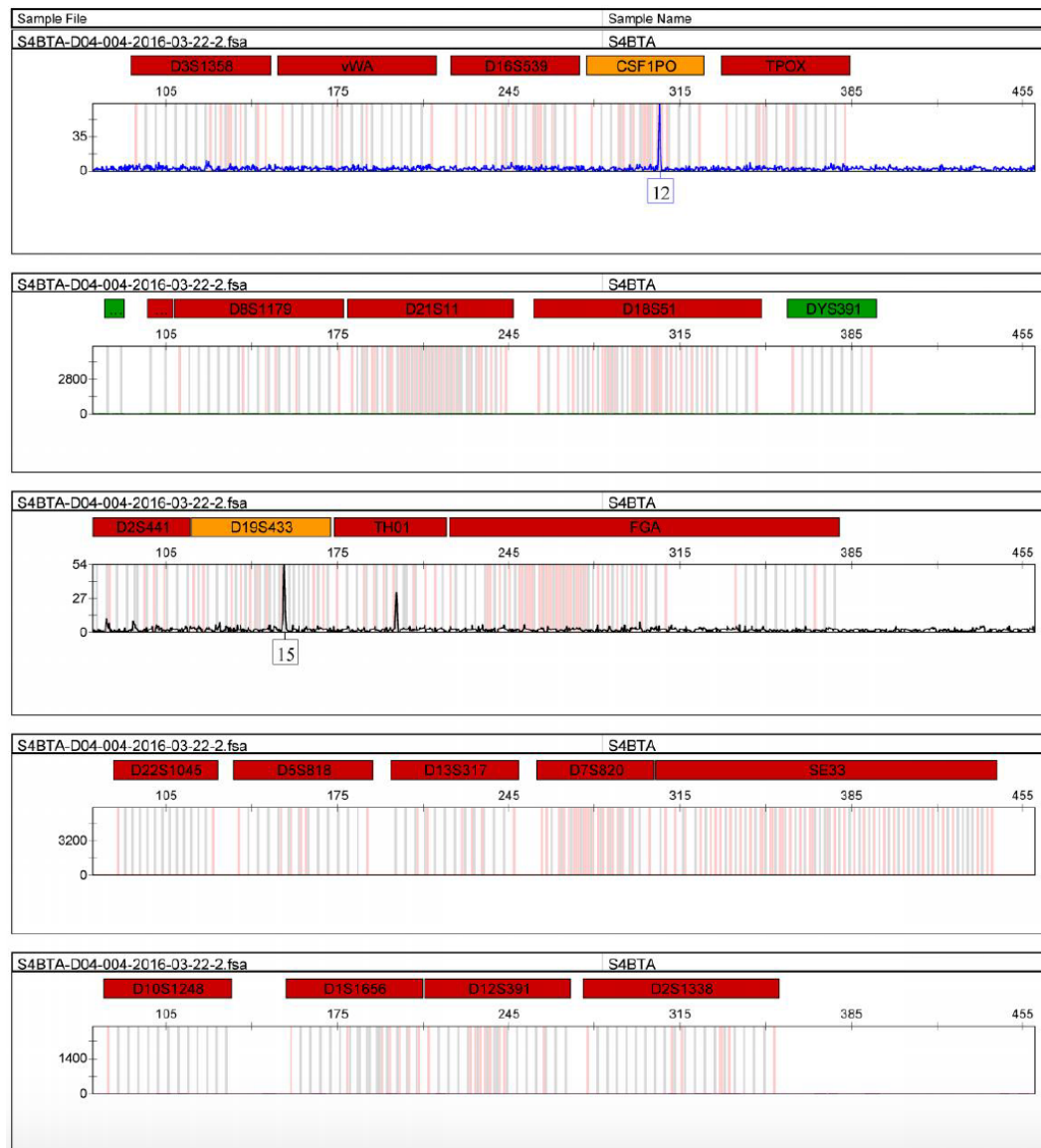
Sample 1
 Pen (Papermate)
 PrepFiler Express BTA™
 10-second injection



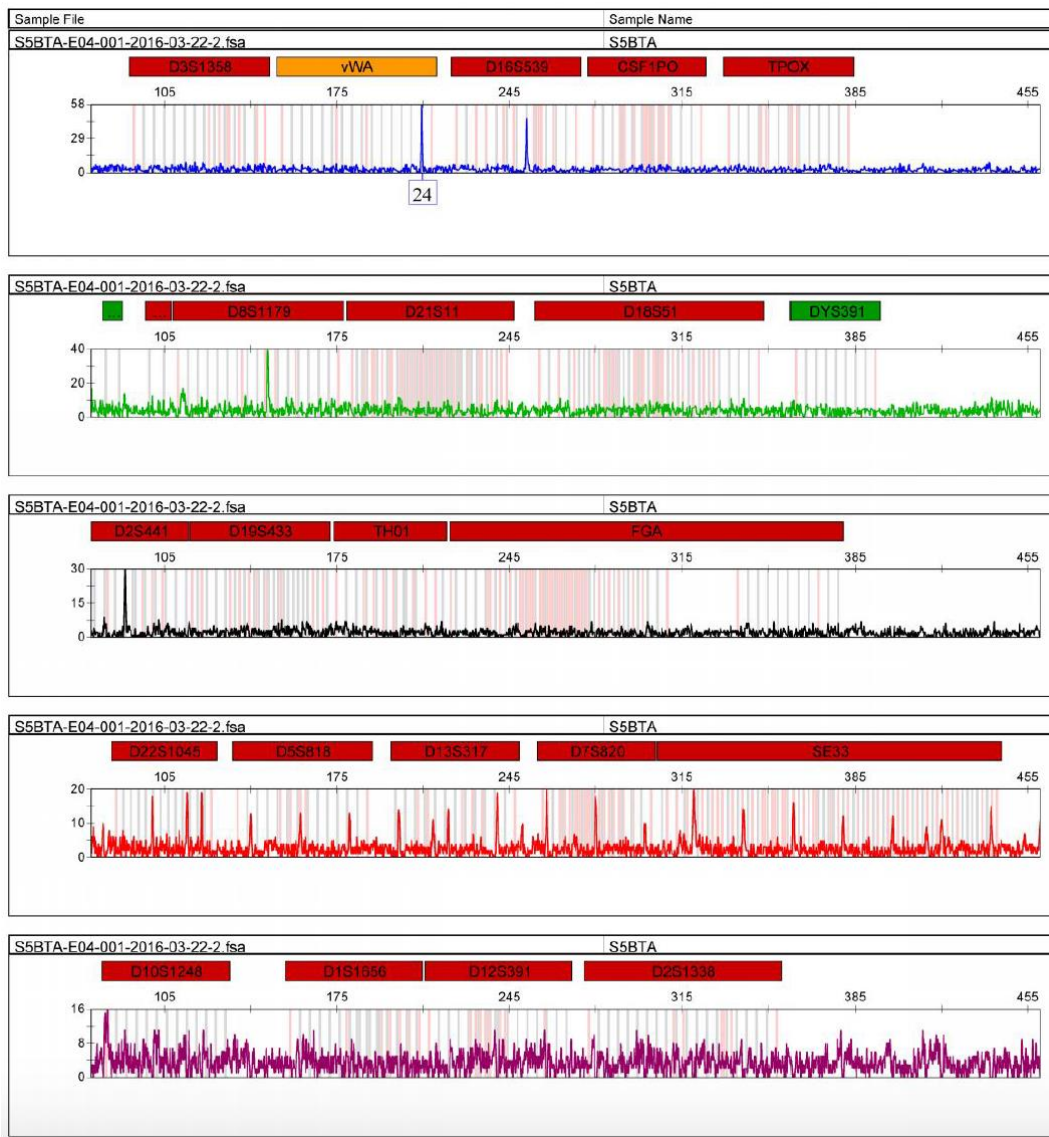
Sample 2
 Wood (Dirty, red paint)
 PrepFiler Express BTA™
 10-second injection



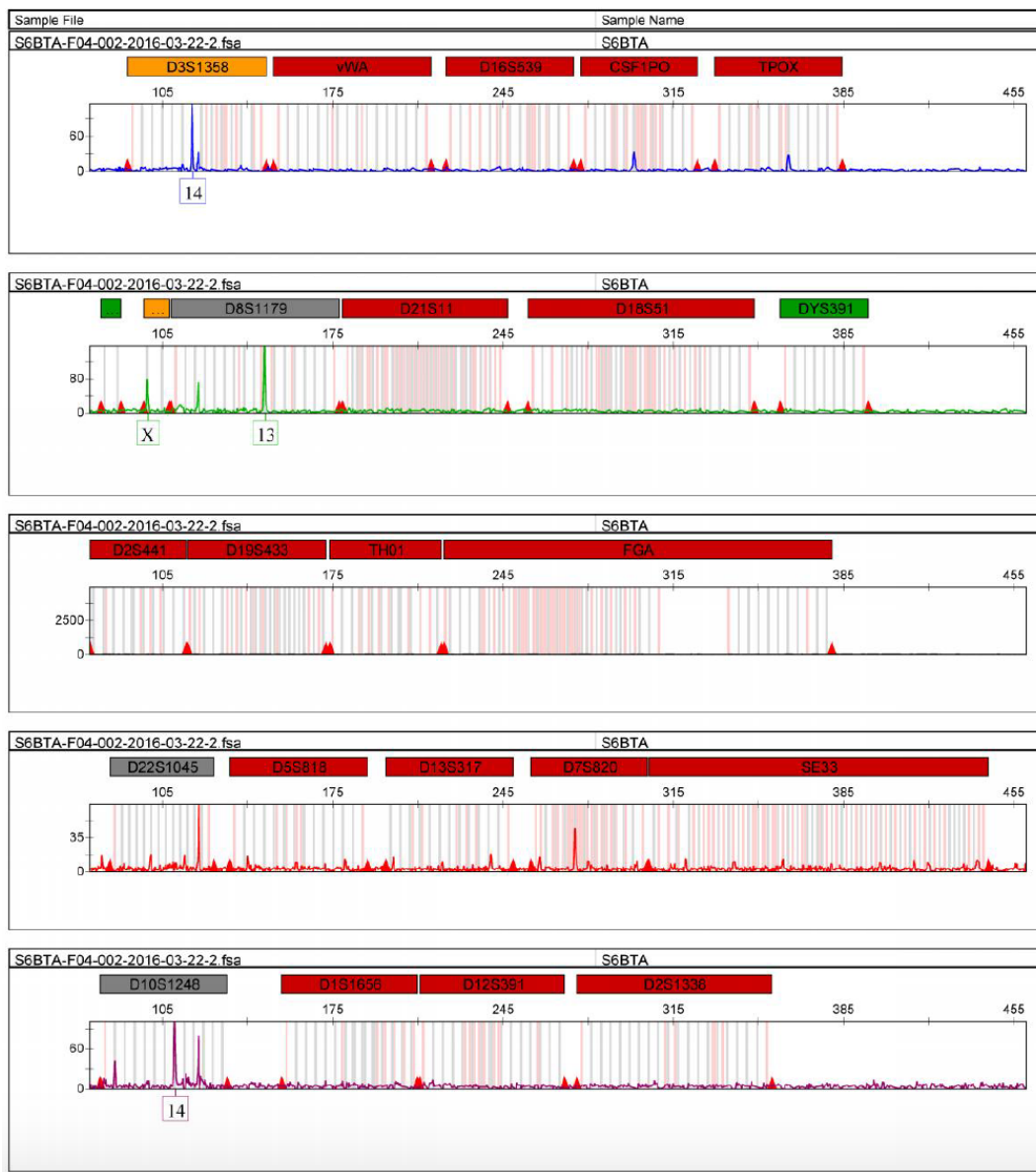
Sample 3
 Back neck of coat
 PrepFiler Express BTA™
 10-second injection



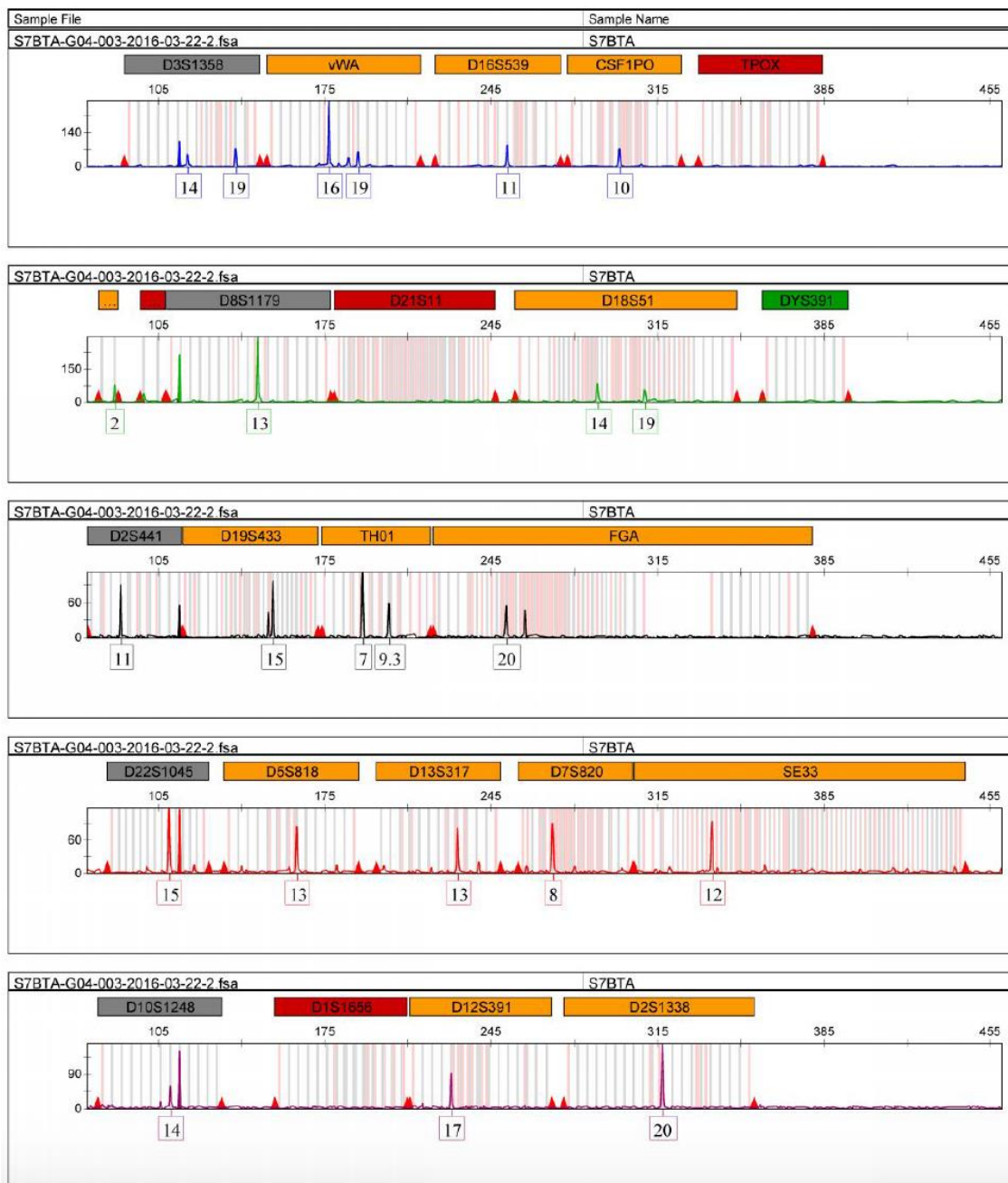
Sample 4
 Pen (Papermate)
 PrepFiler Express BTA™
 10-second injection



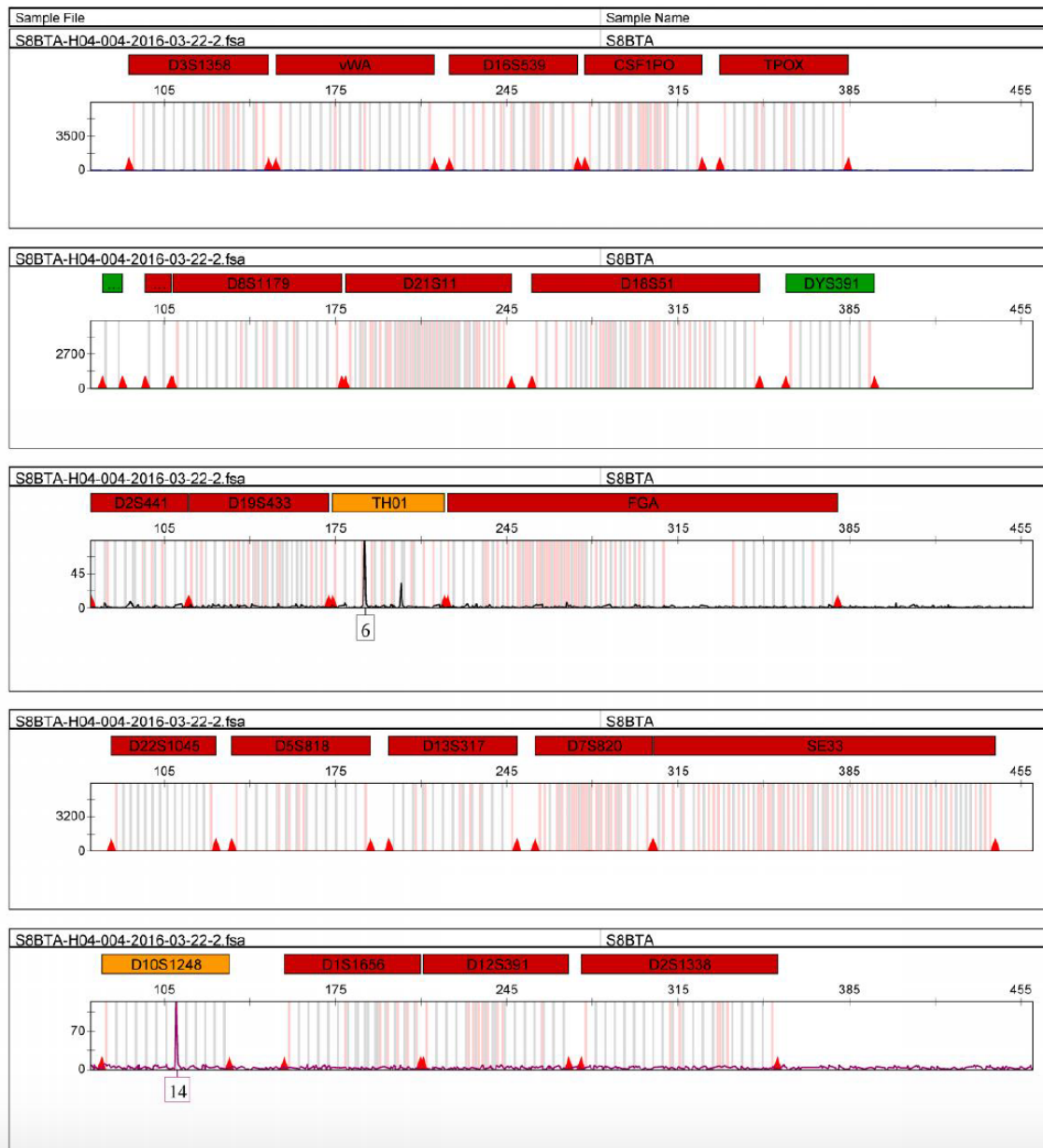
Sample 5
 Wood (Clean)
 PrepFiler Express BTA™
 10-second injection



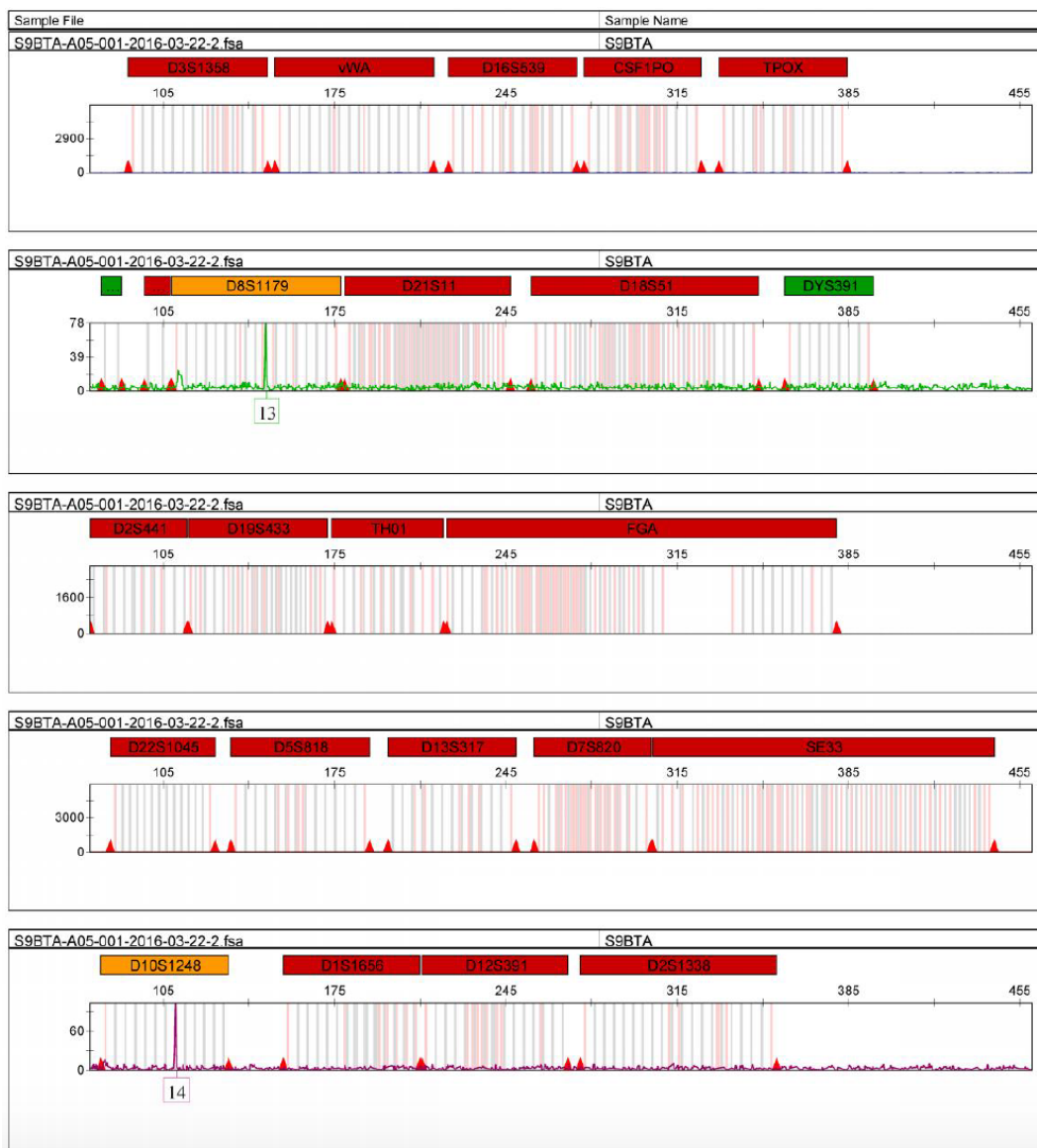
Sample 6
 Scarf
 PrepFiler Express BTA™
 10-second injection



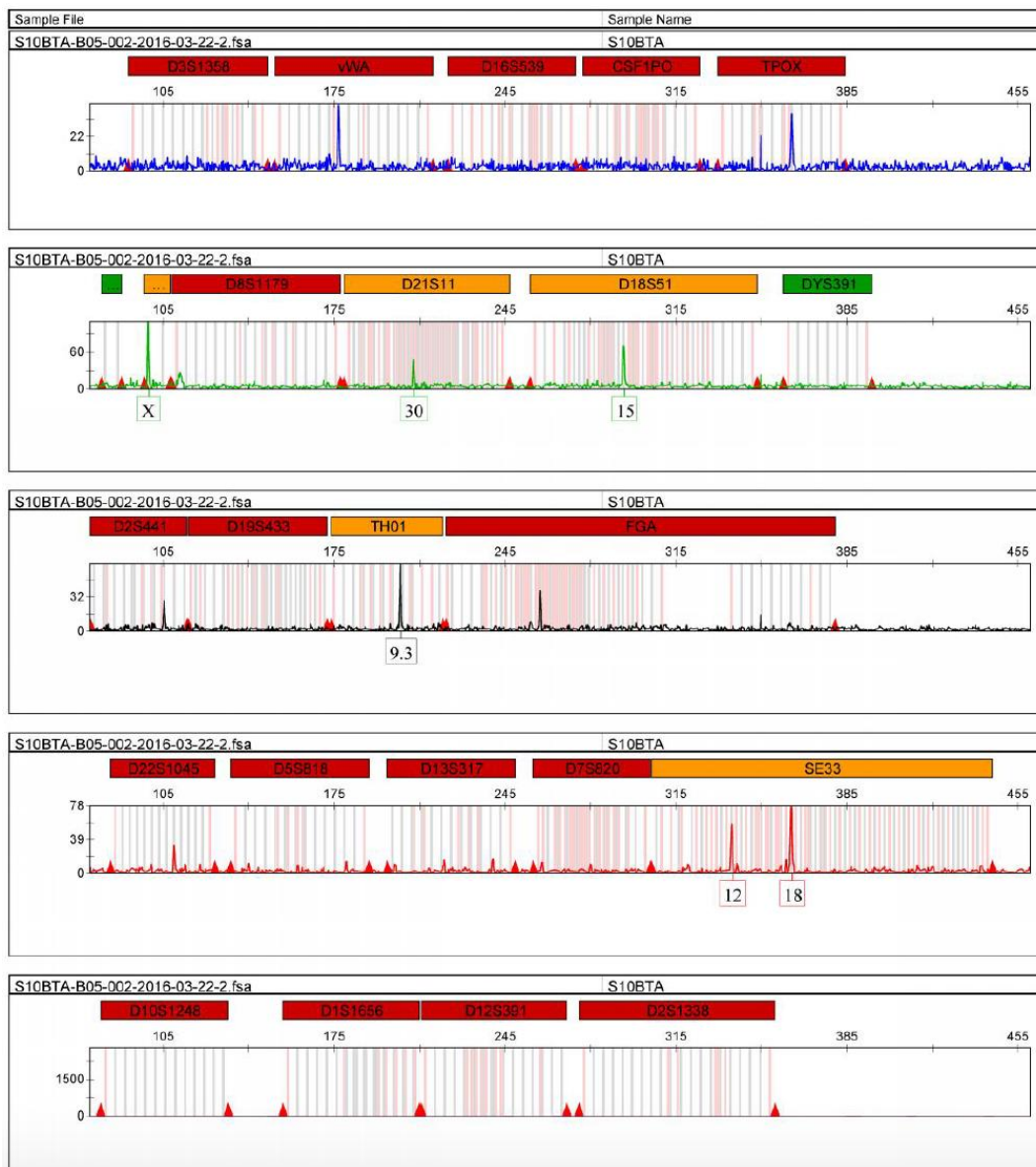
Sample 7
Pencil (Simply Wood)
PrepFiler Express BTA™
10-second injection



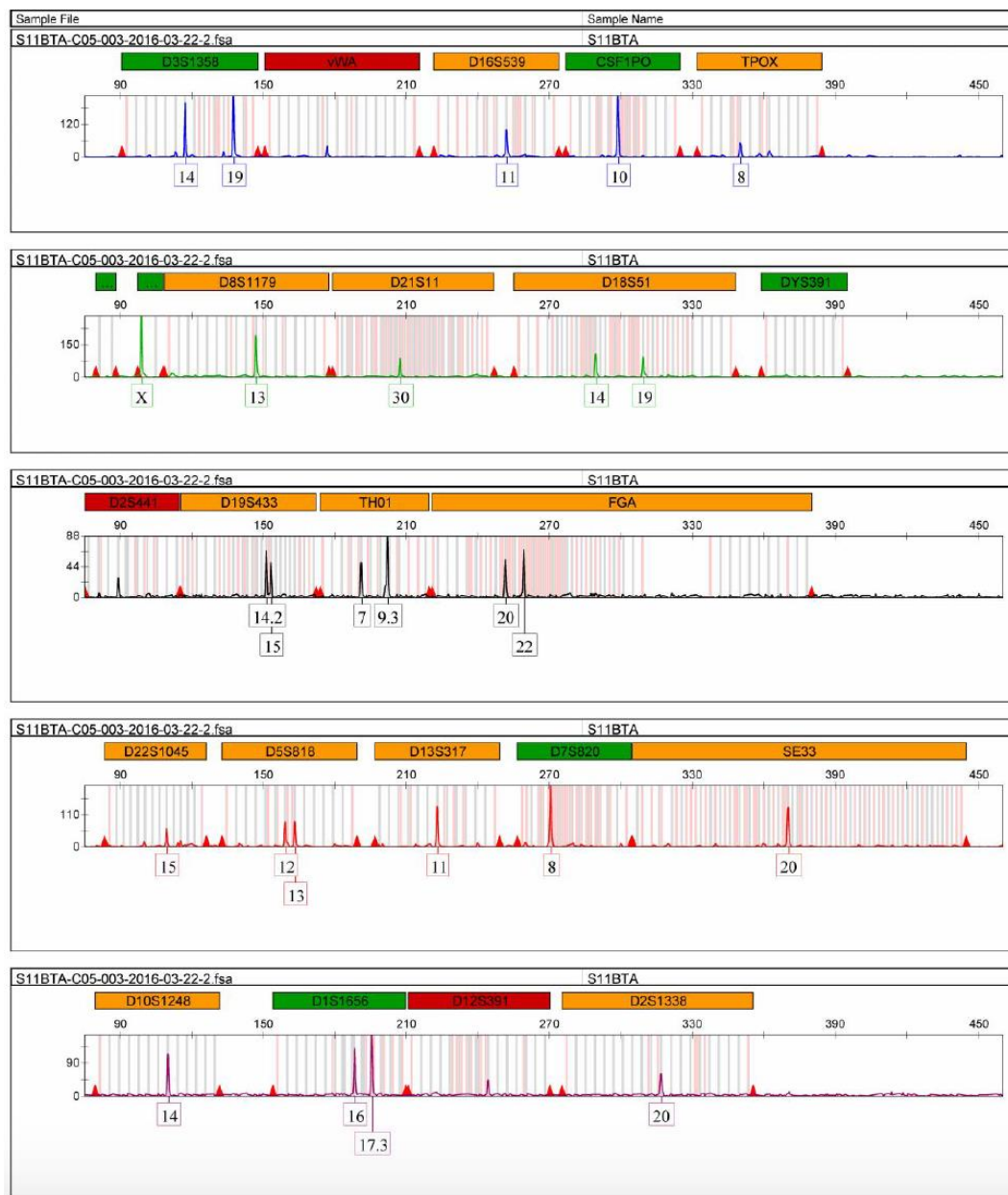
Sample 8
 Wood (Dirty, brown paint)
 PrepFiler Express BTA™
 10-second injection



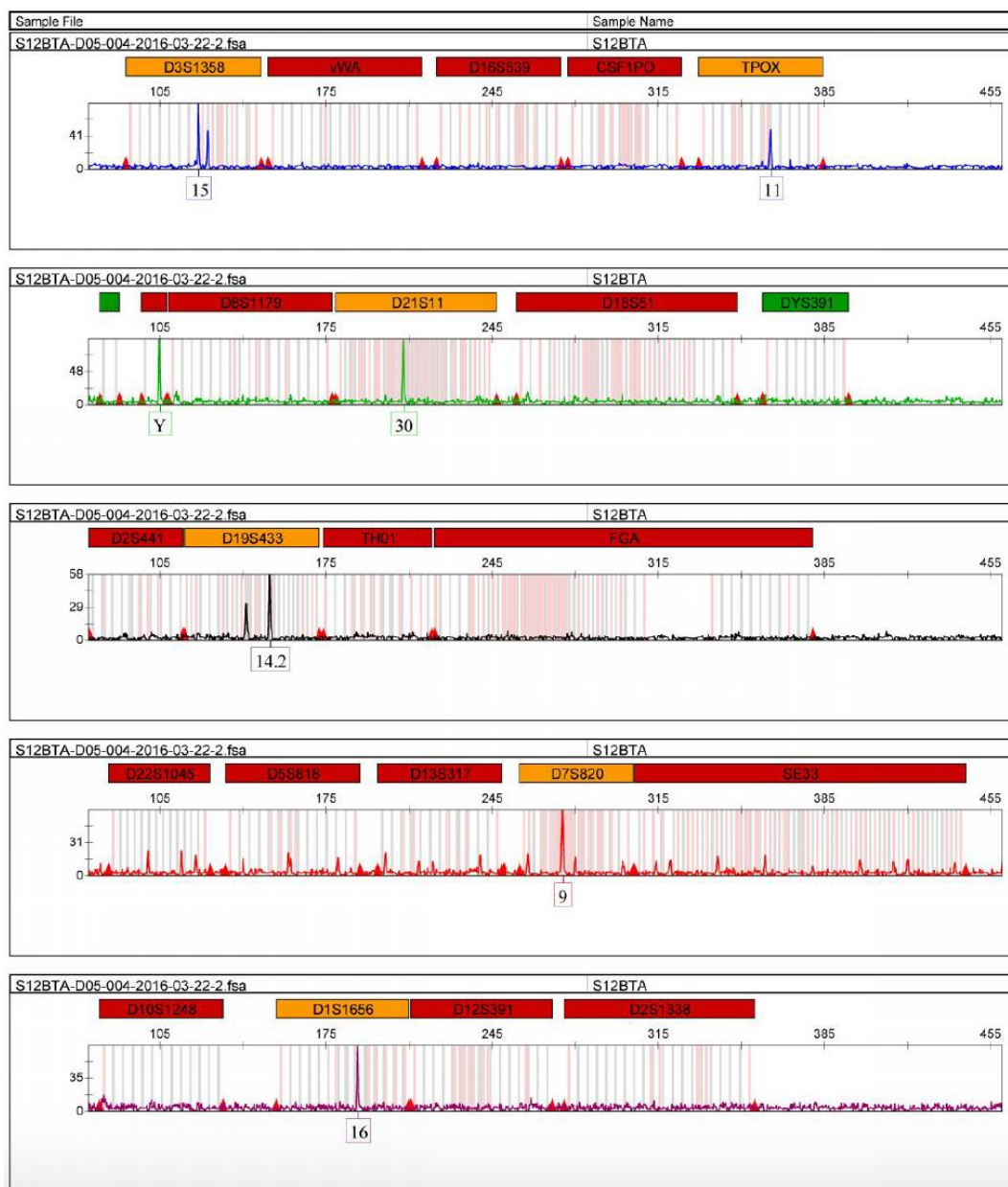
Sample 9
 Lab coat
 PrepFiler Express BTA™
 10-second injection



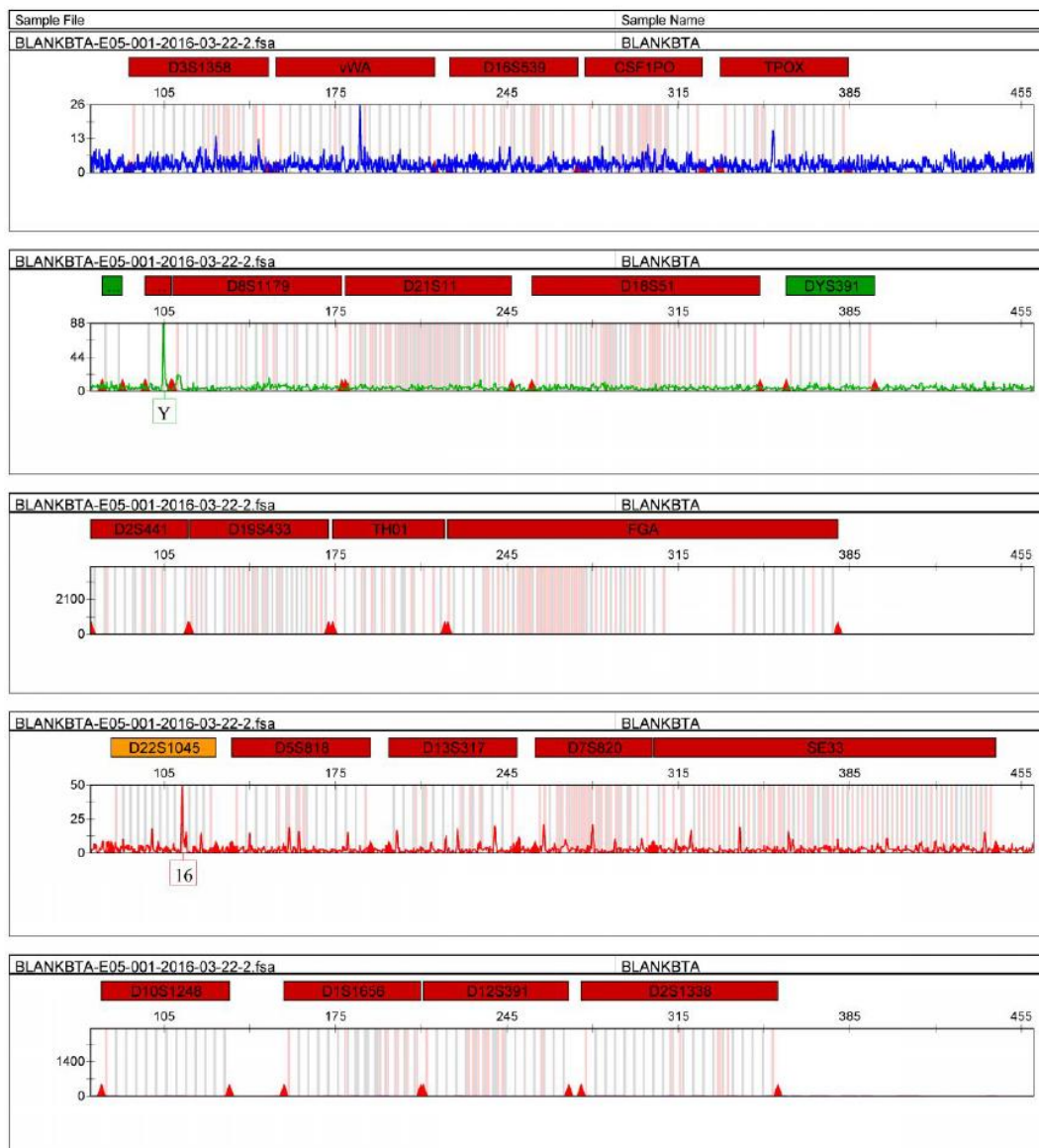
Sample 10
 Pen (Papermate)
 PrepFiler Express BTA™
 10-second injection



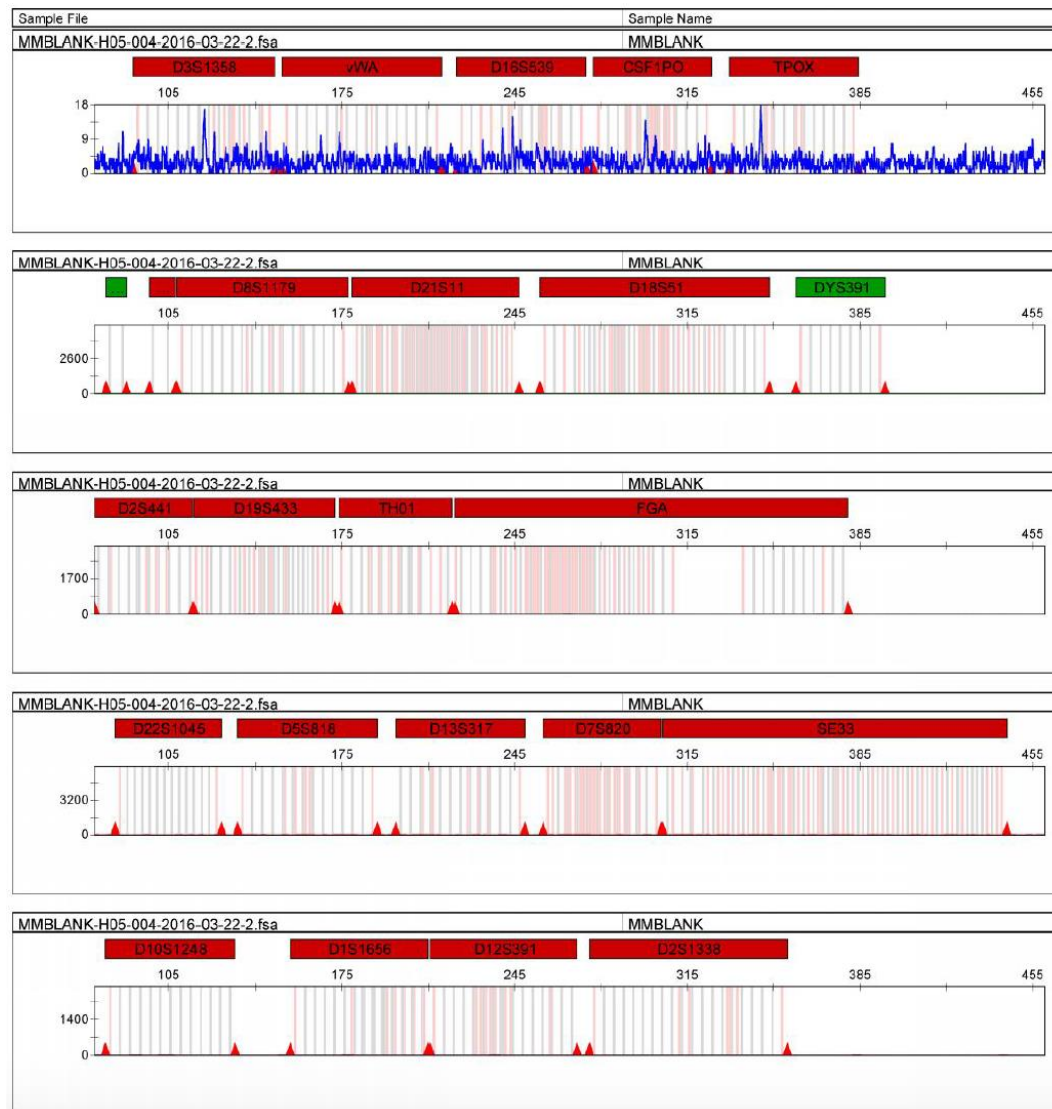
Sample 11
 Wood (Clean)
 PrepFiler Express BTA™
 10-second injection



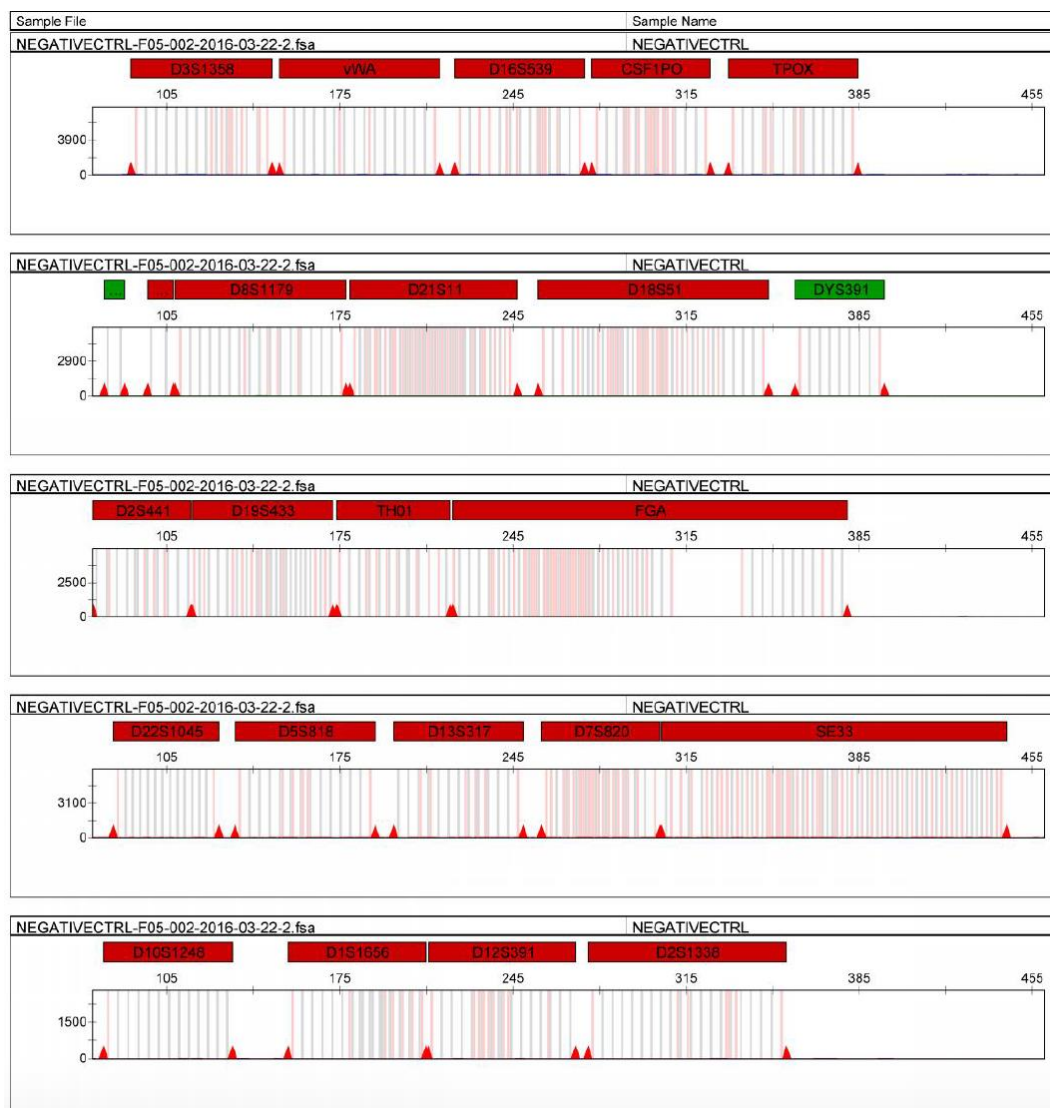
Sample 12
 Inside neck of shirt
 PrepFiler Express BTA™
 10-second injection



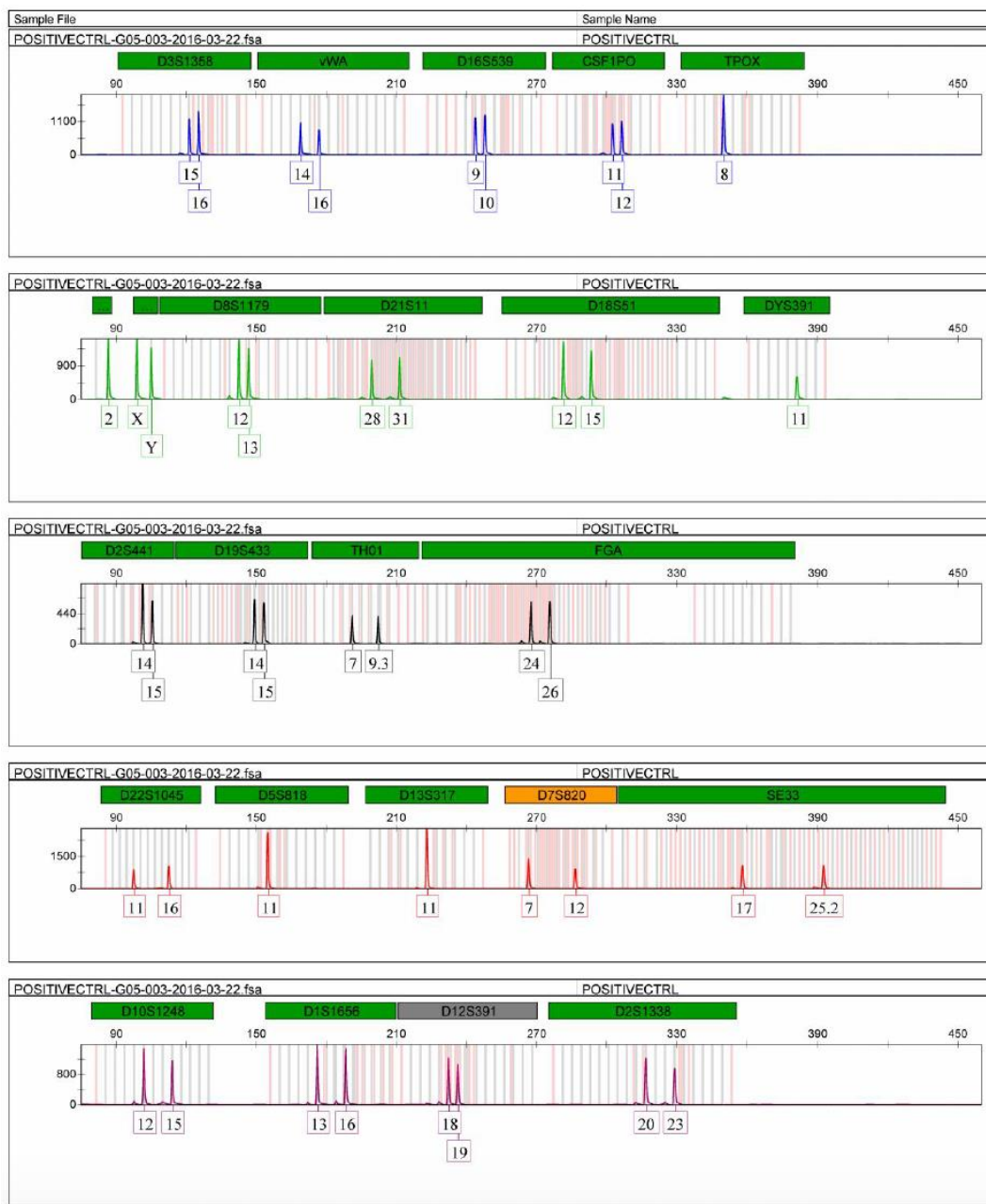
Blank
 Extraction Reagents
 PrepFiler Express BTA™
 10-second injection



Blank/Negative Control
 PCR Reagents
 10-second injection



Negative Control
 PCR reagents
 10-second injection



Positive Control
Control DNA from GlobalFiler kit – DNA 007
1-second injection

REFERENCES

1. van Oorschot RAH and MK Jones. DNA fingerprints from fingerprints. *Nature* 1997; 387: 767.
2. Barash M, A Reshef, and P Brauner. The use of adhesive tape for recovery of DNA from crime scene items. *J Forensic Sci* 2010; 55(4): 1058-1064.
3. Gunnarsson J, H Eriksson, and R Ansell. Success rate of a forensic tape-lift method for DNA recovery. *Prob Forensic Sci* 2010; 83: 243-254.
4. Farash K, EK Hanson, and J Ballantyne. Enhanced genetic analysis of single human bioparticles recovered by simplified micromanipulation from forensic 'touch DNA' evidence. *J Vis Exp* 2015(97).
5. Hall D and M Fairley. A single approach to the recovery of DNA and firearm discharge residue evidence. *Sci Justice* 2004; 44(1): 15-19.
6. Wickenheiser RA. Trace DNA: A review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. *J Forensic Sci* 2002; 47(3): 442-450.
7. *Quality Assurance Standards for Forensic DNA Testing Laboratories*. 2011: Scientific Working Group for DNA Analysis Methods.
8. Adamowicz MS, RD Labonte, and JE Schienman. The potential of cosmetic applicators as a source of DNA for forensic analysis. *J Forensic Sci* 2015.
9. Locard E. The analysis of dust traces. Part i. *American Journal of Police Science* 1930: 276-298.
10. Verdon TJ, RJ Mitchell, and RAH van Oorschot. Evaluation of tapelifting as a collection method for touch DNA. *Forensic Sci Int Genet* 2014; 8(1): 179-186.
11. Lowe A, C Murray, P Richardson, R Wivell, P Gill, G Tully, et al. *Use of low copy number DNA in forensic inference*. in *Int Congr Ser*. 2003. Elsevier.
12. Templeton JE and A Linacre. DNA profiles from fingermarks. *BioTechniques* 2014; 57(5): 259-266.
13. de Bruin KG, SM Verheij, M Veenhoven, and T Sijen. Comparison of stubbing and the double swab method for collecting offender epithelial material from a victim's skin. *Forensic Sci Int Genet* 2012; 6(2): 219-223.
14. Lowe A, C Murray, J Whitaker, G Tully, and P Gill. The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. *Forensic Sci Int Genet* 2002; 129(1): 25-34.
15. Daly DJ, C Murphy, and SD McDermott. The transfer of touch DNA from hands to glass, fabric and wood. *Forensic Sci Int Genet* 2012; 6(1): 41-46.
16. Hansson O, M Finnebraaten, IK Heitmann, M Ramse, and M Bouzga. Trace DNA collection—performance of minitape and three different swabs. *Forensic Sci Int Genet* 2009; 2(1): 189-190.
17. Torre C and S Gino. Epidermal cells on stubs used for detection of gsr with sem-edx: Analysis of DNA polymorphisms. *J Forensic Sci* 1996; 41(4): 658-659.

18. *PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits: User Guide*. 2010.
19. *Quantifiler® Duo DNA Quantification Kit: User's Manual*. 2012.
20. *Applied Biosystems™ Globalfiler™ PCR Amplification Kit: User Guide*. 2015.
21. *DNA Fragment Analysis by Capillary Electrophoresis*. 2014.
22. Vigil BN and TK Kelley-Primo. *Evaluation of three different adhesive tapes for the collection of epithelial cells and the subsequent micro-isolation for PCR analysis*. in *Proceedings American Academy of Forensic Sciences*. 2011.
23. Cupples CM, JR Champagne, KE Lewis, and TD Cruz. STR profiles from DNA samples with “undetected” or low Quantifiler™ results. *J Forensic Sci* 2009; 54(1): 103-107.